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SIGNIFICANCE OF FEED PROTEIN FRACTIONS IN RUMINANT NUTRITION

by

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INTRODUCTION

Research in the field of ruminant nutrition has long emphasized requirements and functions of dietary nutrients. Nitrogenous compounds as well as energy sources, vitamins and mineral elements are well recognized as necessities for animal life. Through proper use of these necessities considerable progress has been made in meat animal production. More complete understanding of the importance of specific dietary nutrients is necessary if progress is to be continued. Nutritional factors which may appear to be minor today may very well be major in the future as more specialized feeding practices are put into operation and as the genetic potentials of our farm animals are approached. Industrial advancements will undoubtedly make available an increased variety of products suitable for use in animal feeds if needs are recognized.

Nutrient requirement studies with ruminants have been complicated to some extent by the perplexing fermentation process which takes place in the rumen. It has become apparent that two separate requirements exist; first, the requirements of the extensive microbial population in the rumen; and second, the requirements of the host animal. Present knowledge indicates that many of the requirements of the host animal are met by microbial synthesis. High quality proteins can be formed, cellulose can be degraded into energy yielding fragments, and certain vitamins can be readily synthesized. Although protein synthesis in the rumen has

been established, quantitative requirements for dietary protein are well recognized.

Little emphasis has been placed on qualitative protein requirements of ruminants. In theory, dietary proteins per se should not be of importance. Rumen microorganisms are capable of degrading feed protein and using nonprotein nitrogen to assimilate into high quality microbial protein. Urea can be successfully utilized by ruminants; however, there seemingly are limits to its use. The specific causes of these limitations are not well known. Certain natural protein feeds have been established as being beneficial to rumen function and animal performance in the presence of adequate amounts of urea nitrogen. Attempts to attribute the beneficial effects of natural protein feeds under such conditions to energy, vitamin and mineral content have been unsuccessful. Sources of energy, vitamins and minerals are well recognized as being important, yet the existence of additional protein feed quality factors has been widely demonstrated. These factors are not well understood at the present time.

Protein quality for ruminants has not been successfully evaluated on the basis of amino acid balance. Factors as yet unidentified seemingly are present in quality protein feeds. If these quality factors could be characterized and their occurrence quantitatively estimated, our understanding of their significance in specific feeding regimens would be greatly advanced. More intensive work to elucidate the specific factors would be stimulated. Such information could perhaps make possible more efficient

use of natural protein feeds and wider use of inexpensive nonprotein nitrogen compounds. Therefore, the purpose of these studies was to determine the nutritional significance of specific fractions of protein feeds. It was hoped that protein quality could be characterized and that the ultimate objective of understanding quality feed factors for ruminants would be advanced.

REVIEW OF LITERATURE

Nitrogen Metabolism in the Rumen

The subject of nitrogen metabolism in ruminant animals has been adequately reviewed from time to time; nevertheless, because of its uniqueness, it is felt that any study concerned with this subject could only appropriately be introduced by a short review of selected works.

Current views of the sequence of events that take place in nitrogen metabolism in the ruminant have been expressed schematically by Chalmers and Synge (28) and more recently by Annison and Lewis (4). This is shown in Figure 1.

Hart and Bentley (49) established that the common feeds fed to ruminants are composed of both protein and nonprotein nitrogen. Pearson and Smith (83) presented experimental evidence showing that nonprotein nitrogen (urea) was first digested to ammonia which in turn was utilized for synthesis of bacterial protein. Their data also indicated this to be the route of ingested proteins. Annison (2) demonstrated that measurable quantities of peptides and amino acids may be present in rumen contents; thus substantiating that proteolysis is the first step in the digestion of proteins in the rumen. Since the classic work of McDonald (67), it has been well established that ammonia is absorbed from the rumen and may amount to a considerable loss of dietary nitrogen if the level of free ammonia in the rumen is in excess (56). Under optimum conditions

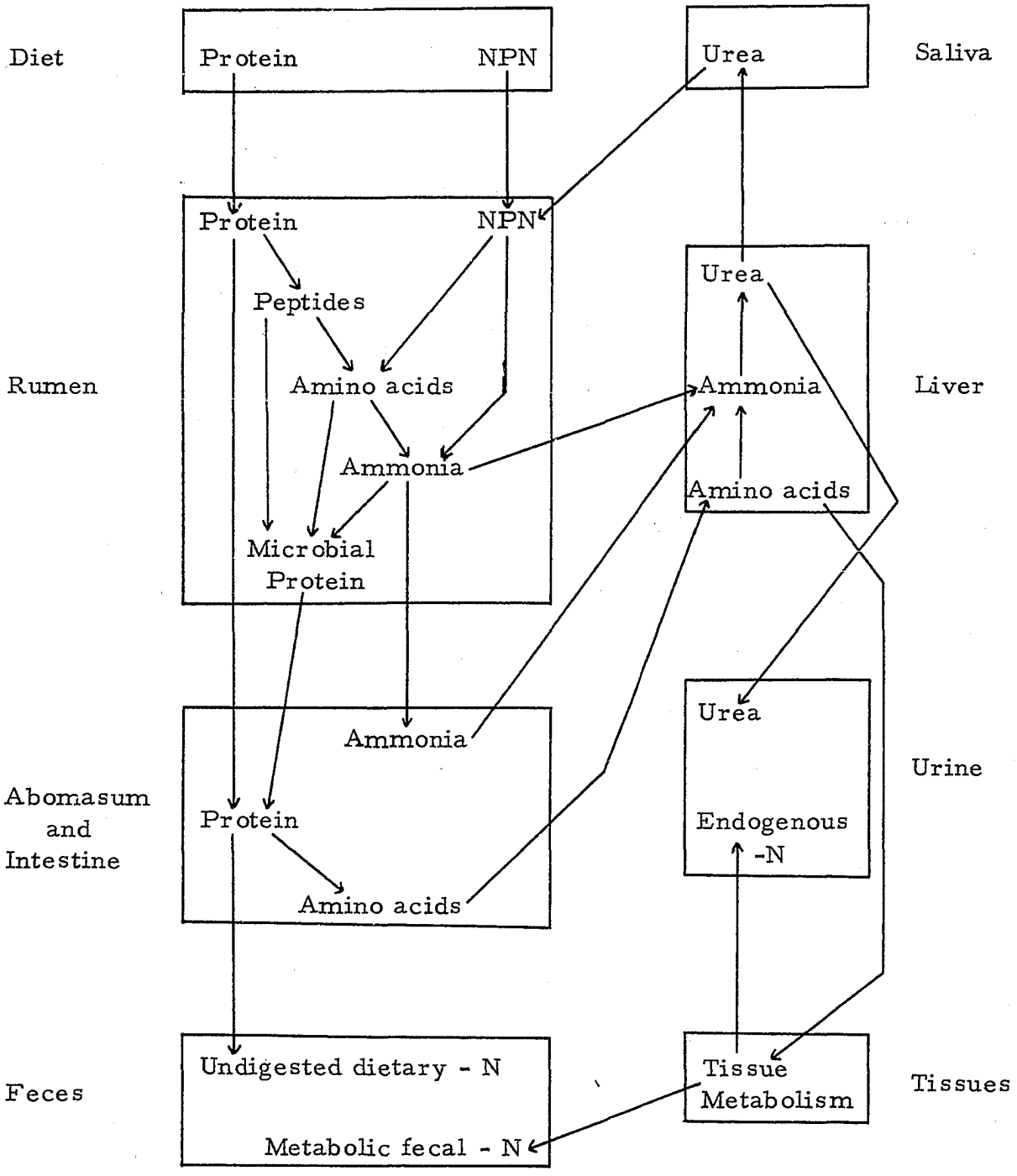

Figure 1. Nitrogen metabolism in the ruminant

much of the ammonia formed in the rumen is used by the microorganisms for protein synthesis. Some of the absorbed ammonia, which passes to the liver and is converted to urea, may return to the rumen via saliva.

El-Shazly (90), Lewis (55) and Looper et al. (61) have shown that amino acids are also broken down in the rumen. Extensive deamination of aspartic acid, glutamic acid, serine, cysteine and perhaps alanine and threonine has been indicated.

McDonald (68) and McDonald and Hall (70) have shown that the extent of protein breakdown in the rumen can differ widely with different proteins. These workers used the specific physical and chemical properties of zein and casein and a series of fistulae in lambs to make quantitative estimations. Under the conditions of their experiments, approximately 60 percent of the administered zein and only 10 percent of the casein passed through the rumen unaltered.

It has been shown indirectly that the tissues of ruminant animals have a requirement for certain amino acids (18); however, these amino acids can be synthesized in the rumen. This ability of rumen microorganisms to synthesize essential amino acids in the process of building microbial protoplasm is reflected in high biological values for nonruminants of both bacterial proteins and protozoal protein (54, 86).

Largely due to lack of evidence to the contrary, it is assumed that, beyond the rumen, nitrogen metabolism in ruminant animals does not differ from nonruminants.

Protein Value of Feeds for Ruminants

Rumen microorganisms, and thus ruminant animals, have the ability to utilize a wide variety of nitrogenous compounds, both protein and non-protein (12, 44, 82, 87). Loosli et al. (63) established that rumen microorganisms were capable of synthesizing all of the amino acids from urea nitrogen which are dietary essentials for nonruminants. The work of Duncan et al. (35) has confirmed this observation. The in vitro studies of Pearson and Smith (83) and McNaught (71) also illustrated the synthetic properties of rumen microorganisms. Some workers interpret these findings, along with other experimental evidence, to indicate that proteins are of equal value for ruminants and protein quality per se is not a factor of significance. There is considerable evidence in the literature to the contrary which indicates that all proteins are not of equal value in ruminant rations; however, there are also numerous reports which have not shown a difference among a wide variety of proteins.

Digestibility and nitrogen balance trials have been used rather widely for studying the value of nitrogen sources for monogastric animals as well as ruminants. An excellent review of the early literature on protein quality for laboratory animals was presented by Boas Fixsen (19). The use of similar methods for studying protein value for ruminants has met with varying degrees of success.

Turk et al. (94) reported the results of digestibility and balance

trials in which the proteins of alfalfa hay and clover hay were fed alone and in combination with the proteins of corn. They noted a difference in favor of alfalfa and particularly a combination of alfalfa and corn. No differences in efficiency of utilization after absorption were evident; however, the biological value of alfalfa protein was increased from 50 to 72 by additions of starch and sugar. In subsequent studies (95) these workers compared the nutritive value of the proteins of soybean oil meal, linseed meal and corn gluten meal. The protein sources were added to a low protein basal ration composed of wheat straw, purified cellulose, starch, sugar and corn oil to make 11 percent of total crude protein. The lambs in this study retained considerably more of the ingested nitrogen from soybean oil meal than from either linseed meal or corn gluten meal. The nitrogen retention of the latter two protein feeds was similar. Miller et al. (76) compared these same three protein feeds when used as supplements for lambs receiving timothy hay or corn stover and corn basal rations. No significant differences among the three feeds were reported.

Miller and Morrison (74, 75) extended this work and found that soybean oil meal, linseed meal, corn gluten meal, casein and dried skim milk furnished proteins of equal values when used as supplements with corn and either timothy hay, corn stover or corn silage as the roughage. Raw soybeans, unextracted soybean flakes, solvent-processed soybean oil meal and "toasted" solvent-processed soybean oil meal were also compared. There was no difference in efficiency with which digested proteins

from these sources were utilized, but the proteins of raw soybeans and unextracted soybean flakes were less digestible. Similarly, Gallup et al. (41) reported no differences among differently processed oil meals.

Miller and Morrison (75) reported that, when urea furnished more than half of the total nitrogen in lamb rations, the nitrogen was less efficiently utilized than when linseed meal or mixtures of linseed meal and urea in ratios greater than 1:1 were used. After reviewing a considerable number of nitrogen balance trials, these workers suggested that there was little or no difference for lambs in the quality of proteins furnished by most feeds.

Ellis et al. (40) studied nitrogen utilization by lambs fed a nitrogen free purified basal ration supplemented with several sources of purified proteins. The biological values of the proteins were determined. In decreasing order they ranked as follows: blood fibrin, soybean protein, casein, gelatin and urea.

Woods et al. (100) reported little differences in digestibility and nitrogen balance between sesame meal and a combination of sesame meal and soybean oil meal when fed to lambs. Two cottonseed meals of different origin and nitrogen solubility were both found to be inferior to the above mentioned protein feeds. In another study (99) these workers compared soybean oil meal, cottonseed meal and sesame meal when fed to lambs at 4, 6 and 8 percent protein levels in semi-purified rations. A total of 90 to 95 percent of the nitrogen intake was furnished by the protein

feed. With respect to protein digestibility, cottonseed meal was inferior to the other two meals. Sesame meal was slightly superior in nitrogen retention. Their data also showed a slight but consistent improvement in crude fiber digestibility in favor of soybean oil meal over sesame meal.

Briggs et al. (20) compared cottonseed meal, soybean oil meal, peanut meal and a mixture of equal parts of the three in a digestion trial with steers. Differences reported among the protein supplements in digestibility of ration constituents were small, and certainly no evidence was presented in favor of a combination of the three.

Johnson et al. (53) compared the utilization of soybean oil meal, casein and urea in nitrogen balance studies with lambs. They reported that the nitrogen in products formed in the rumen from urea was as well utilized in metabolism as was the nitrogen in soybean oil meal and slightly better utilized than casein nitrogen. They noted a consistent increase in cellulose digestion when soybean oil meal was fed. There was a trend in this direction with casein but of less consistency. These workers attributed these differences to available sugars because corn molasses was omitted when soybean oil meal or casein was added. They cited numerous references in support of their work to conclude that the biological value of nitrogen in 10 to 12 percent protein rations generally varies only within a few percentage points of 60.

Hamilton et al. (48) compared urea with either casein, gluten feed

or linseed meal in nitrogen balance studies in paired lamb feeding. They reported no difference between the biological value of urea and casein, only a slight difference between urea and gluten feed in favor of the latter and a sizable difference between urea and linseed meal in favor of linseed meal. It can be noted from their data that the biological value of the nitrogen in the rations containing urea was higher when this compound supplied 47.5 percent of the total nitrogen than when it supplied more than 70 percent of the nitrogen. This work strongly suggests that proportions of dietary nitrogen and microbial protein which actually become available to the animal may very well depend on the composition of the diet.

Hart and Humphrey (51) reported that milk protein was more efficient for milk production than corn or wheat proteins when fed with corn stover. In subsequent trials (50) these workers found that linseed meal and dried distillers' grains provided a more efficient combination of proteins with corn stover than did corn gluten feed; however, no differences among these three concentrates were detected when clover hay was used as the roughage.

Baker (8) reported that distillers' wheat dried grains and urea were inferior to soybean oil meal when fed to wintering heifers on equal nitrogen intake bases. He also reported (9) that, when these nitrogen sources were used as supplements in a steer fattening ration of corn silage and corn, they were of equal value.

Woods et al. (100) reported results of a lamb growth trial in which

two sources of cottonseed meals, sesame meal and a combination of sesame and soybean oil meals were compared. The average daily gain and feed efficiency indicated that sesame meal and the sesame-soybean combination were comparable and were superior to the two cottonseed meals. Noble et al. (79) compared soybean oil meal and urea as supplements to an 8.5 percent protein basal ration for fattening lambs. Soybean oil meal improved rate of gain and feed efficiency while urea alone failed to improve either of these measurements.

Perry et al. (84) and Beeson et al. (10) recently reported results of steer fattening trials in which Purdue A supplement was compared with supplements containing graded levels of urea in place of the soybean oil meal. Their data indicated that the high urea supplements were not as effective as the supplement composed of natural proteins; however, whether this was due to a simple protein effect seems questionable. The supplements were fed daily on a weight basis for equal nitrogen consumption; e.g., 2 pounds of 32 percent Purdue A and 1 pound of 64 percent high-urea supplement. No adjustments were made for minerals, vitamins, alfalfa meal and molasses; therefore, as the urea level increased the level of these other feeds decreased. Supplements of lysine and methionine were also included in these trials, the results of which will be further discussed in another part of this review.

Watson et al. (97) reported a rather extensive study in which comparative values of urea and casein were made with beef animals and sheep.

Increases in carcass constituents were determined and used as the criteria of evaluation. Urea supplementation of low nitrogen basal rations improved carcass composition but not to the extent that resulted with casein supplementation.

Oltjen et al. (81) reported comparisons in purified rations of urea, casein, soybean protein and soybean oil meal. The average daily gains of lambs were 0.31, 0.32, 0.39 and 0.36 pounds, respectively. These workers emphasized the importance of mineral alkalinity of feeds and stressed the high level of potassium carbonate included in their rations.

Belasco (12) has reported high availability of nitrogen from numerous organic and inorganic ammonium salts. He has also compared several protein feeds and urea as nitrogen sources in vitro for rumen microorganisms (11). This work suggested a superiority of urea for promoting cellulose digestion as compared to soybean oil meal, corn gluten meal, linseed meal and cottonseed meal. It also was reported that a combination of urea and each of the protein feeds to make 1:1 ratios of nitrogen improved cellulose digestion without decreasing urea utilization. This indicates a need for readily available nitrogen for efficient rumen fermentation.

It is recognized that some proteins are more extensively attacked than others in the rumen. The work of Annison (2) supports previous findings and further suggests that perhaps distinct chemical or physical properties of bovine albumin, zein and wheat gluten make these proteins

resistant to the proteolytic powers of rumen microorganisms. These three proteins were very slowly attacked in vitro by washed suspensions of rumen microorganisms in contrast to the rapid attack of casein, arachin and soya protein.

The results of McDonald (68) and McDonald and Hall (70) indicated that the insoluble corn protein zein was only 40 percent converted to microbial protein while soluble casein was 90 percent converted. Although these estimations were made under highly experimental conditions, their significance may well be proven to be the key to protein quality for ruminants. Johnson et al. (54) have suggested that feed proteins which are converted to microbial proteins in the rumen have about the same biological value while any protein not utilized by the microorganisms should have a biological value the same as that found for nonruminants.

Protein Feed Fractions Stimulatory to Rumen Function

Requirements of ruminant animals and rumen microorganisms for nitrogen sources have been well recognized. Burroughs and Gerlaugh (22) clearly indicated a need for nitrogen to promote rumen function. When poor quality roughages were supplemented with soybean oil meal, dry matter digestion of high cellulose feeds was markedly improved. Although these workers emphasized the need for available nitrogen in the fermentative processes in the rumen, they suggested that factors in addition to nitrogen may be involved in the responses noted. Since this work was

reported, a considerable number of reports have appeared in the literature to confirm this suggestion.

Many natural substances have been shown to stimulate rumen function in addition to known required nutrients. Various high protein feeds have been recognized as being highly active in this respect, independent of their simple nitrogen content. Although the identity of these factors is unknown, one should be hesitant in referring to them as unknown factors. The possibility exists that these factors may someday be proven to be substances for which the chemical composition is well known. For this reason the term unidentified factors seems to be more appropriate.

Burroughs et al. (25), using an artificial rumen technique, found that roughages considered good quality were digested efficiently without supplementation; however, supplementation of poor quality roughages was necessary. A combination of available nitrogen, minerals and manure extract was required as supplements for the latter. These workers subsequently reported (26) that distillers' dried solubles, linseed meal and soybean oil meal markedly improved cellulose digestion when added to control flasks containing adequate nonprotein nitrogen. Dried skim milk, molasses, corn, wheat bran and cottonseed meal also improved cellulose digestion but to a lesser extent. The three more active feeds also slightly improved digestion when added in addition to minerals and manure. The presence of unidentified factors was suggested, and a need of minerals and available nitrogen for their utilization was indicated.

Burroughs et al. (23) also showed that alfalfa contained unidentified factors which improved digestion of corncob dry matter in steers. Various fractionations suggested that the unidentified factors in alfalfa were soluble in water and perhaps associated in part with the inorganic constituents.

Ruf (88) conducted an extensive study of the properties of the unidentified factors in cow manure extract and in various feeds which stimulate in vitro cellulose digestion by rumen microorganisms. In decreasing order of activity the feeds studied were soybean oil meal, wheat bran, linseed meal, yellow corn, distillers' dark grains, distillers' light grains, dried live yeast, grey shorts and cottonseed meal. Alfalfa meal and bluegrass hay also appeared to contain similar factors. Results obtained with combinations of these feeds indicated no additive effect on in vitro cellulose digestion, thus suggesting that the stimulatory factors contained were similar. Animal by-products were generally of low potency. This work indicated that the factors found in concentrate feeds of plant origin had the following properties: water soluble, heat stable, absorbed on Norite, destroyed by ashing, not absorbed on ion-exchange resins, not removed from solution by various protein precipitation procedures, did not appear to be an amino acid and did not appear to be a B-vitamin.

A lamb growth trial conducted as part of this study and reported by Ruf et al. (89) showed that the addition of 5 percent yeast to a purified ration markedly increased both feed consumption and rate of gain.

Baker et al. (7) further studied the cellulolytic factors present in

distillers' dried solubles by including various preparations of this feed in lamb rations and measuring the digestion of crude fiber. They theorized that the cellulolytic factors contained in distillers' dried solubles were organic in nature and associated with the protein and/or carbohydrate fractions.

McNeil et al. (73) studied the unidentified factor activity of rumen fluid. They suggested that in in vitro studies autoclaved rumen fluid functions importantly as a nitrogen source but also contains additional growth factors. These workers felt that the rumen fluid factors were principally the result of bacterial growth because they were apparently not present in the feeds in their studies prior to ingestion. Garner et al. (42) likewise reported that rumen fluid and certain fermented feeds contained factors stimulatory to cellulose digestion in vitro.

The Ohio station has conducted several studies of the presence and properties of unidentified factors. Bentley et al. (17) reported that the addition of autoclaved rumen fluid, extracts of several good quality roughages, extracts of yeast and molasses favorably stimulated cellulose digestion. An active fraction of rumen fluid was prepared by lead acetate precipitation followed by alcohol extraction of the dried filtrate residue. This material was absorbed on Norite A and eluted, but the eluate was less active than the original rumen fluid. B-vitamins, purines and uracil additions along with the eluate fraction increased digestion above the eluate alone.

Further in vitro studies reported by Bentley et al. (16) indicated that the cellulolytic factor activity of rumen fluid was contained in the volatile fatty acid fraction and could be attributed primarily to valeric acid content. Caproic, isobutyric and isovaleric acids, in addition to valeric acid, were active in stimulating cellulose digestion and ammonia utilization. It was also noted that biotin and para-aminobenzoic acid were required for maximum cellulose digestion in these studies. A valeric acid-vitamin combination duplicated but was not identical to unidentified factors in certain natural feeds. In light of the results of el-Shazly (90) which indicated that the origin of such fatty acids in the rumen was from deamination of amino acids, it was reasonable to assume that the unidentified factors in feeds were present in the form of corresponding amino acids. Evidence in this respect was presented by Dehority et al. (34) when they reported that valine, proline, leucine and isoleucine were cellulolytically active when added at levels comparable to the content of yeast and alfalfa extracts and a casein hydrolysate. Other workers have failed to support these observations (49, 93).

Bentley et al. (15) reported a slight improvement in steer gains when valeric acid, biotin and para-aminobenzoic acid were added to a fattening ration composed of ground ear corn, corncobs, corn gluten meal and urea. These factors failed to give a response when a practical ration containing soybean oil meal was fed. In a concurrent digestibility trial with lambs fed a purified ration, the addition of these factors increased dry matter

intake but did not appear to influence cellulose, protein or total dry matter digestibility. These workers suggested that unidentified factors increase rate of digestion in the rumen while percent digestion is unaffected.

In a recent paper, Wegner and Foster (98) reported that 20 of 90 bacterial isolates from the rumens of cows on high roughage rations would not grow unless rumen fluid was included in the medium. A mixture of fatty acids commonly found in the rumen was found to effectively replace rumen fluid in the medium. Isovalerate would replace both the rumen fluid and the acid mixture while normal valerate would not.

Hall et al. (46) reported that several B-vitamins stimulated cellulose digestion by washed suspension of rumen microorganisms; however, no combination of B-vitamins was found which would stimulate cellulose digestion as much as the stimulation from yeast extract. This suggested the presence of unidentified factors in addition to the B-vitamins. Woods and Tillman (101) reported that a mixture of B-vitamins improved both gain and feed consumption when added to a purified ration fed to lambs. Novak et al. (80) reported a procedure for concentrating a factor from alfalfa which influenced pyridoxine assay organisms. Liuzzo et al. (59) reported that this concentrate was active in promoting cellulose digestion in the artificial rumen.

Although it has been established that rumen microorganisms can synthesize all of the amino acids considered essential for nonruminants, the literature contains many reports of stimulation in microbial activity

and animal performance with amino acid supplementation. Loosli and Harris (62) were among the first to report a favorable response with methionine supplementation. Urea utilization by lambs was increased when a high level of methionine was added to the ration. Lofgreen et al. (60) likewise reported that methionine supplementation increased nitrogen retention in lambs fed urea as the major nitrogen source. In their experiments a combination of methionine and urea was equal in promoting nitrogen retention to linseed meal while urea alone was inferior.

Noble et al. (79) added methionine to fattening rations for lambs and noted a slight increase in rate of gain when this amino acid was added to a high protein ration containing urea. Methionine additions had no effect in a low protein ration or in a ration containing soybean oil meal as the protein source.

In studies of optimum protein levels for lambs, Griffith et al. (43) reported significantly increased gains when methionine was added to a 11 percent protein ration. A comparable improvement in gain resulted when the protein level was increased by additional soybean oil meal. The response produced by methionine in some rations has been linked to the sulfur content. Methionine appears to be a very effective source of sulfur for ruminants (1).

Trenkle (93) tested 21 amino acids individually, each at several concentrations, in vitro and noted improvement in cellulose digestion only with alanine, proline and methionine. Methionine was shown to increase

gain and feed consumption of lambs fed a purified ration, but it was not effective in rations of natural feeds.

Morris and Wright (78) summarized the results of a series of experiments in which several protein feeds were compared as nitrogen sources for milk production. Amino acid analyses of each of the proteins fed were reported. They suggested that, when minimum quantities of proteins are fed to producing milk cows, an inadequacy of either lysine or tryptophan leads to a marked decrease in milk yield and marked increases in urinary nitrogen. Ellis et al. (39) more recently reported that the lysine level of rumen contents following feeding of several purified proteins could not be correlated with differences in nitrogen balance and biological value of the proteins. They were able to show a correlative significance between tryptophan level in the rumen and both nitrogen balance and biological value. It was suggested that the ability of rumen microorganisms to synthesize microbial protein from some isolated nitrogen sources was inadequate to meet the needs of the animal for certain amino acids.

In a recent report by Hale et al. (45) lysine supplementation was suggested as beneficial for both cattle and lamb gains. These workers reported that the feeding of 10 grams of lysine daily to steers on a high protein ration (11 percent) increased gains but was without effect in a lower protein ration. Likewise, a sizable increase in gain was noted when lambs were fed a pelleted ration containing 900 grams of L-lysine per ton.

The Purdue station has also reported (10, 84) improved gains of steers when 10 grams of lysine were fed daily. They noted that as the urea level in the supplement increased the response from lysine increased. Methionine was without effect in these studies when fed at an equal level. They also did not get a response when these two amino acids were combined. It thus appears that the amino acid fraction of protein feeds in ruminant rations may be of significance under certain conditions.

Role of Ammonia and Factors Affecting Its Utilization

Much evidence has accumulated suggesting that ammonia is the major intermediate in nitrogen digestion and metabolism in the rumen. Ammonia has been shown to be formed by microbial attack of proteins and nonprotein nitrogen alike.

Various studies have shown that the rate of ammonia production from different proteins differs markedly. McDonald (69) has linked this property of a protein to its solubility. He compared the rate of ammonia production from casein, gelatin and zein when administered directly to the rumen and found that casein and gelatin were quickly hydrolyzed and deaminated to ammonia but zein was not. Chalmers et al. (27) confirmed these findings and further found that heat treating a protein was influential. By subjecting casein to high temperatures, less rapid ammonia production was noted and better nitrogen utilization resulted. They also showed that when casein was administered via fistula directly into the duodenum, it

was equally digestible but better utilized than when administered into the rumen. This suggested that casein was attacked too rapidly and part of the nitrogen was lost by absorption of free ammonia. Heated casein was more slowly attacked and apparently little nitrogen was lost by ruminal absorption.

Annison et al. (3) determined the rate of ammonia production when three different protein sources as supplements to a basal hay ration were fed to sheep. Ammonia production was greatest from groundnut meal, intermediate from herring meal and only slight from corn products, viz., ground corn and corn gluten meal. Chalmers and Synge (29) reported that herring meal produced less ammonia than casein when added as supplements to a high roughage, low protein basal diet. They likewise noted a difference in nitrogen balance and lamb growth in favor of herring meal when this type ration was fed. They were unable to detect a difference when a ration containing a higher proportion of starch to roughage was fed. They attributed the differences in the former to be a result of extensive ruminal ammonia formation and thus loss of nitrogen from casein.

These English workers (3, 27, 29) surmised that the rate of ammonia production from a protein may be inversely related to its value in certain high roughage ruminant rations. They suggested that, in a preliminary investigation of a ration, determining ammonia production in a fistulated animal may be a time-saving step. These workers acknowledged, however, that differences between extent of ammonia formation appeared to be of

little significance when feeds were fed containing proportions of starch to protein usually found in fattening rations.

Annison (2) continued these studies and compared a wider variety of proteins. The breakdown and ammonia formation of casein, bovine albumin, arachin, zein, wheat gluten and soya protein were compared *in vitro*. Casein, arachin and soya protein exhibited considerable breakdown while the others were less extensively attacked.

Ellis *et al.* (40) studied the rate of ammonia produced *in vivo* from several purified nitrogen sources. Their data indicated little difference in ammonia production when gelatin, casein, blood fibrin or soybean proteins were fed as the nitrogen source in purified rations containing high levels of starch and dextrose. They did report a marked increase in rumen ammonia when urea was fed as the nitrogen source. Correlatively, urea also had the lowest biological value in their trials.

The work of el-Shazly (90), which established that amino acids are decomposed to ammonia in the rumen, also suggested that this decomposition is increased as the content of soluble proteins in the diet is increased. Rumen fluid taken from lambs receiving a casein supplemented diet more readily decomposed mixture of amino acids than rumen fluid taken from lambs receiving hay only. Lambs receiving fresh grass were similar in this property to the casein fed lambs. Perhaps the total number of active microorganisms was increased (21).

The early work relating the need of readily available carbohydrates

for ammonia utilization, particularly from nonprotein nitrogen sources, has been discussed in detail in reviews by McNaught and Smith (72) and Owen (82).

Arias et al. (5) observed that urea utilization in vitro was markedly improved by the addition of several energy sources as readily available carbohydrates. Pierce (85) likewise interpreted increased wool production in sheep with the addition of starch to a low protein ration containing urea as evidence of improved urea utilization. Mills et al. (77) analyzed rumen contents at intervals after feeding high urea rations and found that protein content increased markedly when starch additions were made to a timothy hay-urea basal ration.

Belasco (13) used an in vitro artificial rumen to show that starch increased ammonia utilization to a greater extent than xylan, pectin or glucose; however, the largest improvements were noted with supplements of starch and cellulose combined. More recently, Lewis and McDonald (57) presented the results of an extensive study of the interrelationships of casein and several carbohydrates during rumen fermentation in vivo. They confirmed in vitro results by showing that the carbohydrates reduced ruminal ammonia concentrations arising from degradation of casein. Starch and levan were most effective in achieving this response. Glucose and xylan were somewhat effective while cellulose had little effect.

The data of Lewis and McDonald (57) also indicate that a small supplement of casein to a low quality diet stimulates the rate of

fermentation of the added carbohydrates as determined by fatty acid formation. Similarly, Burroughs et al. (24) reported that starch increased the protein supplement needs for efficient roughage digestion.

These findings have been interpreted to indicate that some readily available energy is needed by rumen microorganisms to assimilate rapidly produced ammonia into protoplasmal protein during the time cellulose is being broken down to a utilizable form.

Evidence thus suggests that ammonia represents an important intermediate in rumen digestion of dietary proteins and nonprotein nitrogen alike. McDonald (69) has summarized the role of ammonia in ruminant digestion as leading to two opposing nutritional tendencies. First, it is undesirable from the standpoint that high quality dietary proteins may be degraded to ammonia which can be absorbed and lost. On the other hand, it is very desirable from the standpoint that low quality dietary proteins and nonprotein nitrogen compounds such as urea, which are of little value to the tissues, can be degraded and synthesized into high quality microbial protein. Apparently certain carbohydrates play an important part in limiting this undesirable tendency.

EXPERIMENTAL AND RESULTS

Significance of Soluble Nitrogen

Considerable information has appeared in the literature and was reviewed on comparisons of protein and nonprotein nitrogen feeds. There is sufficient information to support that all nitrogen sources are not of equal value in ruminant rations. Evidence has suggested that simple physical properties of proteins may influence this feeding value. Some workers have indirectly shown that the soluble nitrogen content of protein feeds may be of significance. Two opposing hypotheses have been advanced. First, the English workers have surmised that a protein of low nitrogen solubility is of higher value for ruminants than a protein of high nitrogen solubility. They base their views on data which indicate that high soluble nitrogen sources are very rapidly converted to ammonia in the rumen, and through ruminal absorption a sizable amount is lost. On the other hand, an insoluble nitrogen source is not readily available to rumen nicroorganisms and rumen function, particularly synthesis of high quality microbial protein, is curtailed.

Few detailed experiments designed to specifically test the relation of nitrogen solubility and protein value have been reported. This work, therefore, was initiated to determine the significance of soluble nitrogen fractions of feed proteins. Soluble nitrogen content of several feeds was determined using three different solvents; rate of ammonia production

from some of these feeds was measured; their influence on in vitro cellulose digestion was compared; and the values of low and high-soluble nitrogen feeds in lamb fattening rations were studied.

Methods and results

Soluble nitrogen content of various feeds The purpose of this preliminary experiment was to determine the soluble nitrogen content of a variety of natural feeds and purified proteins. Dilute sodium hydroxide (.02N), distilled water and rumen fluid were used as solvents. Several workers (30, 64, 65) have reported a correlation between nitrogen soluble in dilute sodium hydroxide and protein value of cottonseed oil meal for poultry. Water is often thought of as a universal solvent, and the solubility of nitrogen in rumen fluid should be indicative of solubility in the rumen.

The rumen fluid used as a solvent was prepared in the following manner. Rumen ingestum was obtained from a fistulated steer, strained through several layers of cheese cloth and centrifuged in a Servall angle centrifuge to remove suspended feed material. The liquor was then passed through a Sharples super-centrifuge to remove the microorganisms. This supernatant was autoclaved at 15 pounds pressure for 30 minutes and cooled to room temperature before being used.

The nitrogen extraction technique reported by Lyman et al. (64) was used with some modifications. A one-gram sample of finely ground feed

was placed in a 250 milliliter Erlenmeyer flask containing three glass beads. One hundred milliliters of the solvent were added along with two drops of actyl alcohol to control foaming. The flasks were stoppered and shaken on a Fisher-Kahn mechanical shaker at room temperature for 55 minutes. They were allowed to set for 5 minutes and the liquid decanted. The suspended residue was separated from the liquid by centrifugation or filtration. Fifty milliliters of the supernatant liquid were pipetted into a Kjeldahl flask, and the total nitrogen in the aliquot was determined by the usual Kjeldahl procedure. The total nitrogen in each feed was determined, and the soluble nitrogen was expressed as the percent of the total nitrogen.

Heat is recognized as a factor which influences nitrogen solubility by denaturing proteins. Samples of some of the feeds were heated prior to extraction so that the effect of heat treatment on nitrogen solubility could be appraised. These samples were heated in a drying oven at 110° C for 24 hours.

The nitrogen soluble in the respective solvents of several feeds and purified proteins as determined is given in Table 1. Solubilities of nitrogen in dilute sodium hydroxide generally were higher than in the other two solvents. The solubilities in water and rumen fluid were found to be similar for most of the feeds analyzed. Purified casein was an exception in this respect, being much more soluble in rumen fluid than in distilled water. Heat treating soybean oil meal, linseed meal and corn markedly

reduced nitrogen soluble in all solvents. Soybean oil meal, heated soybean oil meal, linseed meal, corn gluten meal and purified casein, soy protein and zein were selected as representatives of nitrogen solubility patterns indicated for further study.

Table 1. Soluble nitrogen content of feeds, using .02 N NaOH, H_2O and rumen fluid as solvents

	Percent total N soluble		
	.02N NaOH	H_2O	Rumen fluid
Soybean oil meal	81	16	19
Heated soybean oil meal[a]	30	11	10
Linseed meal (solvent)	68	39	45
Heated linseed meal (solvent)[a]	42	15	14
Distillers' dried solubles	53	27	48
Corn gluten meal	32	11	13
Purified casein	98	2	81
Purified soy protein	99	2	7
Purified zein	99	0	3
Corn	71	18	23
Heated corn[a]	30	13	15
Alfalfa hay	36	--	28

[a]Heated in drying oven at 110° C for 24 hours.

Relation of soluble nitrogen and rate of ammonia production by rumen microorganisms The role of ammonia in rumen metabolism has been reviewed. It is generally accepted that ammonia is the major intermediate between feed protein breakdown and microbial protein synthesis in the rumen. The level of free ammonia in the rumen is believed to be indicative of the rate of feed protein attack and breakdown; however, it seems reasonable to assume an inverse relation to microbial synthesis. Experimental evidence has been reviewed which suggests that rate of ammonia production is influenced by the solubility of the protein being degraded, thus a link of protein attack with nitrogen solubility is indirectly suggested.

The objective of this study was to gather additional information concerning the relation of soluble nitrogen and rate of ammonia production. The rate of ammonia production from a limited number of the proteins for which nitrogen solubility determinations had been made in the preceding experiment was measured. An *in vitro* technique was developed for this purpose and use in a preliminary experiment. Additional information was obtained *in vivo* using fistulated lambs.

For the *in vitro* experiment rumen contents were obtained from a fistulated steer which was fasted 16 hours prior to sampling. The ingestum liquid was strained through four layers of cheese cloth into prewarmed thermos bottles. This rumen liquor was immediately taken to the laboratory, saturated with carbon dioxide gas and adjusted to a pH of

7 with sodium carbonate. It was then divided into 200 milliliter portions in 250 ml. Erlenmeyer flasks containing equal nitrogenous levels of the protein sources studied. Each flask was fitted with a rubber stopper containing inlet and outlet glass tubes. The flasks were incubated at 39° C with a continuous inflow of carbon dioxide gas. Small aliquots were taken at hourly intervals. Ammonia was separated by the procedure of Conway (32) using potassium carbonate in the outer chamber and 0.2N sulfuric acid in the inner chamber. Nessler's reagent was used to colorimetrically determine the ammonia. The protein samples were added in amounts to provide 320 milligrams of nitrogen per 200 milliliters of rumen liquor.

The in vivo ammonia experiments were conducted using lambs fitted with permanent rumen fistulae. In the first experiment the lambs were maintained on 2 pounds daily of a mixed ration composed of 47 percent corn, 40 percent alfalfa hay, 7 percent molasses, 5 percent soybean oil meal and 1 percent salt. The lambs were fasted 24 hours before the beginning of the experiment. Fifty-gram samples of casein, soy protein or zein were administered. This was done by adding enough water to the protein sample to make a slurry, warming the slurry to 39° C and forcing it into the rumen through the fistula with a drenching gun. An equal volume of distilled water was administered in the same manner to control lambs. Samples of rumen contents were taken before and at intervals after administration. The samples were strained through 8 layers of

cheese cloth, and the ammonia in the liquor was separated and quantitatively determined as stated above.

In the second in vivo experiment the lambs were maintained on a mixed ration composed of 25 percent oat straw, 15 percent corn cobs, 41 percent corn, 8 percent molasses and 11 percent soybean oil meal. In the first part of this experiment the lambs were fasted 24 hours followed by feeding 225 grams of regular soybean oil meal or heated soybean oil meal (110° C for 24 hours). In the second part the lambs were fasted 12 hours followed by feeding 1.5 pounds of the maintenance ration containing either regular soybean oil meal or heated soybean oil meal. The same procedures for sampling and ammonia determination as described above were followed.

The results of the in vitro ammonia experiment are presented in Table 2. The values presented represent milligrams ammonia nitrogen per 100 milliliters rumen liquor above the ammonia at the same time of sampling in a control flask. The control flask containing only rumen liquor showed a slight rise in ammonia during the incubation period, but ammonia above the control should represent that arising from an added nitrogen source. Casein, soy protein, soybean oil meal and linseed meal were converted to ammonia at about an equally rapid rate, with a tendency for casein to exceed the other three. In contrast, the ammonia levels in the flasks containing zein, heated soybean oil meal and corn gluten meal showed only a slight increase in ammonia.

Table 2. Rate of ammonia formation in rumen liquor in vitro from different protein sources

(Values expressed as mg. NH_3-N per 100 ml. above control)

	Incubation time (hours)			
	1	2	3	4
Casein	3.8	7.0	11.4	12.5
Soy protein	3.0	5.5	7.6	10.2
Zein	0.1	2.9	0.5	0.9
Soybean oil meal	4.2	7.3	9.3	10.2
Heated soybean oil meal	2.5	2.5	2.5	3.0
Linseed meal	3.0	6.9	7.4	8.0
Corn gluten meal	2.2	3.1	3.2	1.6

Table 3 summarizes the results of ammonia formation in vivo from some of these same protein sources. The levels of rumen ammonia formed from casein far exceeded the other proteins compared. As was indicated in the in vitro experiment, ammonia formed from zein was essentially nil. The ammonia formed from soy protein was somewhat intermediate, being lower in comparison to casein than was observed in the in vitro experiment.

Similar to above, soybean oil meal was apparently much more rapidly degraded to ammonia than heated soybean oil meal. Although the

sharp decline in rumen ammonia when heated soybean oil meal was administered seems questionable, the lack of change during the second and third hours after treatment would support a conclusion that this protein feed was very slowly converted to ammonia. It should be emphasized that only the proteins were administered in the above treatments, and also the lambs did not have access to water during the period of observations. The latter may account for the tendency of the ammonia level to rise in the controls even when the lambs were fasted 24 hours before sampling was begun.

When a complete ration was fed as in trial 2 of experiment 2, a steady decline in ammonia was observed. No differences were noted between the high soluble nitrogen and the low-soluble nitrogen rations. Fifty percent of the protein in these rations was furnished by regular soybean oil meal and heated soybean oil meal, respectively. These rations did contain a large amount of readily available carbohydrates, 41 percent corn and 8 percent molasses.

Comparison of protein feeds of determined nitrogen solubility as nitrogen sources for in vitro cellulose digestion by rumen microorganisms

Results of the preceding experiments had suggested some relation between nitrogen solubility and rate of ammonia production. If this relation is indicative of microbial attack and breakdown of proteins, then protein feeds of differing nitrogen solubility should affect variable responses in in vitro cellulose digestion by rumen microorganisms when they are

Table 3. Rate of ammonia formation in rumen liquor *in vivo* from different protein sources

(Values expressed as mg. NH_3-N per 100 ml. change from 0 hour)

Experiment Number	Protein Source	Time after treating (hours) 1	2	3	4	5	6	8
1	Control[a]	-1.2	-2.1	2.4	--	2.4	4.8	--
	Casein[b]	9.9	16.2	23.0	23.9	21.9	27.1	
	Control[a]	1.3	4.2	4.9	5.5	4.9	6.2	6.2
	Soy protein[b]	3.6	4.8	5.9	4.3	5.0	5.5	7.3
	Control[a]	-1.6	--	0.6	1.2	-2.4	4.0	4.8
	Zein[b]	-0.4	-3.6	-1.4	-0.7	-0.7	2.8	3.2
2	Trial 1							
	Control[c]	--	.6	3.0	3.5	--	8.5	11.1
	SBOM[c]	--	7.7	10.4	11.5	--	13.0	9.2
	HSBOM[c]	--	-10.8	-12.8	-12.3	--	-8.2	-7.8
	Trial 2							
	10% HSN ration[c]	-0.1	-5.9	-7.5	-5.2	-10.2	--	--
	10% LSN ration[c]	-1.3	-5.8	-7.4	-9.6	-9.9	--	--

[a]Average of duplicate samples from one lamb.

[b]Average of duplicate samples from three lambs.

[c]Average of duplicate samples from two lambs.

compared as nitrogen sources in the artificial rumen. The purpose of this experiment was to compare some of the feed proteins in this respect.

The washed cell suspension artificial rumen technique of Cheng et al. (31) was used in this experiment. The only modification was that digestion time was increased from 24 hours to 30 hours. Rumen liquor was obtained from a 1400 pound steer fitted with a permanent rumen fistula. This animal was maintained on a daily ration intake of 4 pounds corn, 2 pounds soybean oil meal, 6 pounds alfalfa hay and 4 pounds oat straw. Samples were taken 5 to 7 hours postprandial. Treatments were triplicated in each trial. The composition of the nutrient medium is given in Table 4. Each of the nitrogen sources studied was finely ground and added to the digestion tubes at the rate of 4 milligrams nitrogen per 20 milliliters inoculum.

The Tukey test as described by Snedecor (92) was used to determine a difference required for significance at the 0.05 probability level for comparison of treatment means in each trial.

The results of this in vitro experiment are summarized in Table 5. The influence of the various protein feeds on cellulose digestion tended to parallel the extent of ammonia formation indicated in the preceding experiment. Heated soybean oil meal and corn gluten meal, which had been shown to be only slightly converted to ammonia, were apparently not utilized as nitrogen sources for in vitro cellulose digestion. They supported no greater cellulose digestion than when no nitrogen was added.

In contrast, regular soybean oil meal, linseed meal, casein and soy protein were highly effective as nitrogen sources as shown in comparison to urea. These four above mentioned proteins had been shown to be extensively converted to ammonia.

Effect on lamb performance of reducing nitrogen solubility by heat treating ration ingredients Experimental results in preceding phases of this work suggested that the value of a protein source for rumen function may be related to the soluble nitrogen content of the source. Heat treatment was shown to markedly reduce nitrogen solubility as well as reducing both ammonia formed and value of the protein feed for supporting in vitro cellulose digestion by rumen microorganisms. Although no apparent difference was observed in rate of ammonia production in vivo following ingestion of high carbohydrate rations containing either regular or heated soybean oil meal, the former was superior in stimulating cellulose digestion and in rate of ammonia production when they were administered alone. Information was desired concerning the significance of soluble nitrogen in lamb fattening rations.

Differences noted between various feeds were equally as large as differences between regular and heated soybean oil meal; nevertheless, if the nitrogen solubility of one feed was markedly reduced artifically and compared with the original, this would be a more critical test of nitrogen solubility significance than comparing two different feeds. Two different feeds, while differing in nitrogen solubility, may also differ in

Table 4. Composition of nutrient medium

Constituent	Amount (gm./liter)
Cellulose[a]	5.00
KH_2PO_4	0.30
$Na_2HPO_4 \cdot 7H \cdot 0$	0.60
$NaHCO_3$	1.75
KCl	2.00
NaCl	2.00
$CaCl_2$	0.275
Na_2SO_4	0.150
$MgSO_4$	0.075
$FeSO_4 \cdot 7H_2O$	0.038
$CuSO_4 \cdot 5H_2O$	0.001
$CoCl_2 \cdot 6H_2O$	0.001
$MnSO_4 \cdot 5H_2O$	0.0002
$ZnSO_4 \cdot 7H_2O$	0.00004

[a]Solka-floc, a purified wood cellulose.

other essential nutrients.

For the above reasons regular and heated soybean oil meal and corn were compared in the first lamb trial. They were each fed at three protein levels in low quality roughage rations. The heated soybean oil meal and heated corn were prepared by placing them in shallow trays and heating in a forced air oven at 110 - 120° C for 24 hours. Upon removal from the oven, the heated feeds were spread in thin layers and allowed to cool and moisture equilibrate with the air.

A 2x3 factorial arrangement of six treatments was followed, two

Table 5. Influence of various protein feeds as nitrogen sources for *in vitro* cellulose digestion by rumen microorganisms

Treatment	Percent cellulose digested	
	Trial 1[a]	Trial 2[b]
Control (No N)	13.0	12.9
Urea	17.8	23.0
Soybean oil meal	20.2	28.5
Heated soybean oil meal	10.7	14.4
Corn gluten meal	12.2	15.1
Linseed meal	19.2	31.9
Casein	--	25.5
Soy protein	--	27.7

[a]A difference (D) of 3.1 required for significance at P= .05 level for comparison of treatment means.

[b]D= 6.9

levels of soluble nitrogen and three levels of proteins. The compositions of the rations according to protein level are given in Table 6. Lambs on treatments 1, 2 and 3 were fed the 7, 10 and 13 percent protein rations, respectively, containing low soluble nitrogen heated soybean oil meal and heated corn. Lambs on treatments 4, 5 and 6 were fed the 7, 10 and 13 percent protein rations, respectively, containing high soluble nitrogen

Table 6. Compositions of rations fed to lambs in Trial 1

Ration Ingredients	Parts per 100		
	7% protein	10% protein	13% protein
Oat straw	25.0	25.0	25.0
Corn cobs	14.5	14.5	14.5
Cracked corn	49.0	40.6	31.7
Soybean oil meal	2.5	11.0	20.0
Molasses	8.0	8.0	8.0
Sodium chloride	0.5	0.5	0.5
Limestone	0.2	0.2	0.3
Dicalcium phosphate	0.3	0.2	--

1,000 units of vitamin A and 125 units of vitamin D were added per pound of each of the rations

regular soybean oil meal and regular corn. Adjustments were made for lower moisture contents of the heated feeds.

Thirty-six western crossbred wether lambs were randomly assigned to the six treatments. The average initial weight of the lambs was 70 pounds. They were vaccinated for enterotoxemia and sore mouth and drenched with phenothiazine prior to the start of the experiment. The lambs were individually fed by placing them in individual feeding crates for two 3-hour periods each day. The lambs had access to water and

block salt while not in the crates.

The results of this 70 day feeding trial are presented in Table 7. The statistical analysis can be found in Table 24 of the Appendix. The results were statistically analyzed by analyses of variances according to Snedecor (92) and tested for significance by the F test. There were no significant treatment effects. The average daily gain of the lambs on the three treatments receiving the low soluble nitrogen rations containing heated ingredients was equal to that of the lambs receiving regular ration ingredients. Likewise, the lambs fed the low protein ration gained equally as well as the lambs fed higher protein rations. Feed required per pound of gain paralleled rate of gain, differences being within normal ranges of variation.

Comparison of protein feeds of differing nitrogen solubility in semi-purified rations for lambs Reduced nitrogen solubility by heat treating ration ingredients failed to affect lamb performance in the previous trial. The results of that trial were unique in that no effect was noted when the protein level was increased above 7 percent. If the value of a protein was reduced by heat treatment, one might expect the largest effect at a marginal protein level. A fair test of the protein value of a specific protein feed should be made when that protein feed comprises the major portion of the total ration protein. Although nitrogen solubility was also decreased in corn by heat treating, the real significance of soluble nitrogen of soybean oil meal was not actually

Table 7. Effect on lamb performance of heating protein ingredients to reduce nitrogen solubility

	70 day ave. daily gain of 6 lambs (lbs.)	Ave. daily feed consumption (lbs.)
7% protein, low soluble N[a]	.34	3.25
10% protein, low soluble N[a]	.29	3.09
13% protein, low soluble N[a]	.36	3.22
7% protein, high soluble N[b]	.36	3.46
10% protein, high soluble N[b]	.34	3.44
13% protein, high soluble N[b]	.31	3.46

[a]Composed of heated soybean oil meal and corn (110° C for 24 hours).

[b]Composed of regularly processed soybean oil meal and corn.

measured in the low protein rations in the preceding trial. Soybean oil meal provided only 16 percent of the total protein in the 7 percent rations and only 48 percent of the total in the 10 percent rations.

The purpose of this experiment was to compare protein feeds of different nitrogen solubilities in marginal protein lamb rations to which they furnished the major portion of the total protein. A semi-purified ration was formulated and soybean oil meal, heated soybean oil meal, linseed oil meal and corn gluten meal were compared as protein sources.

Approximately 90 percent of the total ration protein (8%) was provided by these protein feeds. The compositions of the rations are shown in Table 8.

Twenty-four lambs were used in this experiment with an average initial weight of 80 pounds. They were vaccinated for enterotoxemia and sore mouth prior to the start of the experiment. The lambs were group fed the soybean oil meal ration (ration 1) for 7 days, after which time they were randomly alloted to the 4 treatments and placed in individual pens. The lambs were changed to their respective experimental rations during an additional 7 day period. Thus, a 14 day preliminary period preceded the actual experimental period. The individual pens were bedded with wood shavings and the lambs had access to feed and water at all times.

Table 9 shows the lamb performance data of this 42 day feeding trial, and statistical analyses of these data are summarized in Table 25 of the Appendix. Treatment means were tested for significance by Duncan's multiple range test (36). Generally, these lambs performed excellently as can be seen from the rapid rate of gain and the quantity of feed consumed daily. These are unusually good for lambs fed this type of ration. Similar to the results of the preceding lamb trial, little difference was noted between regular and heated soybean oil meal. Slightly higher gain and feed consumption resulted when heated soybean oil meal was fed; however, the differences were well within normal variation.

Table 8. Compositions of semi-purified rations fed to lambs in Trial 2

Ingredient	Ration			
	1	2	3	4
Corn cobs	20.0	20.0	20.0	20.0
Cellulose[a]	20.0	20.0	20.0	20.0
Corn dextrose	14.4	14.4	11.7	14.3
Corn starch	13.3	13.3	10.6	13.2
Corn oil	3.0	3.0	3.0	3.0
Mineral mixture[b]	6.3	6.3	6.3	6.3
Syrup[c]	5.0	5.0	5.0	5.0
Vitamin mixture[d]	1.0	1.0	1.0	1.0
Soybean oil meal	17.0	--	--	--
Heated soybean oil meal[e]	--	17.0	--	--
Linseed meal	--	--	22.4	--
Corn gluten meal	--	--	--	17.2

[a]Solka-floc, purified wood cellulose.

[b]Composition of mineral mixture given in Table 22 of Appendix.

[c]Composed of 3.75 pounds sucrose and 1.25 pounds water.

[d]Composition of vitamin mixture given in Table 23 of Appendix.

[e]Heated in forced air drying oven at 110° C for 24 hours, adjustments were made for decreased moisture.

The linseed oil meal fed lambs performed similarly to the soybean oil meal fed lambs. The performance on corn gluten meal was much inferior to the performance on the other protein feeds. The lambs gained at a much slower rate and consumed less feed when the corn gluten meal was fed as the protein source. This suggests that the protein in corn gluten meal is of less value than the protein in soybean oil meal and in linseed oil meal.

Table 9. Effect on lamb performance of different protein feeds as nitrogen sources in semi-purified rations

Treatment	42 day ave. daily gain of 6 lambs (lbs.)	Ave. daily feed consumption (lbs.)
1. Soybean oil meal	.55	3.52
2. Heated soybean oil meal	.61	3.67
3. Linseed meal	.60	3.69
4. Corn gluten meal	.28	2.75

Discussion

Protein feeds have been shown to differ in soluble nitrogen content. It was also established that heat treatment reduced nitrogen solubility in the limited number of feeds so studied. It is evident that nitrogen soluble in any one solvent is not necessarily indicative of the solubility in other solvents; therefore, one should be hesitant in saying that a feed protein is highly soluble or slightly soluble without specifying the solvent. Lyman

et al. (64), Chang et al. (30) and Mann et al. (65) reported correlations with similar success between nutritive value of cottonseed meal samples for chicks and nitrogen soluble in .02N NaOH, .5N NaCl and 6N HCl. These workers suggested the use of a nitrogen solubility index as a laboratory method of evaluating protein feeds. Eagle et al. (38), however, reported a poor correlation between biologically evaluated protein quality of cottonseed meal with rats and nitrogen solubility in dilute alkali. Woods et al. (100) showed no difference in growth or nitrogen balance in lambs when high soluble nitrogen and low soluble nitrogen cottonseed meals were compared. These workers did not state the solvent used or the quantitative nitrogen solubility.

The results of the ammonia experiments with individual proteins are in agreement with other reported studies. Several workers have shown that casein is rapidly degraded to ammonia in the rumen (2, 29, 69). Annison (2) illustrated that purified soy protein was extensively attacked by rumen microorganisms in vitro; however, it was not as rapidly converted to ammonia as casein. Purified zein and various cereal glutens have been reported to be less extensively attacked and only slowly converted to ammonia (2, 3, 69). Marked reduction of ammonia formation by heat treating a protein has also been reported (27).

No definite relation can be drawn from these experiments between rate of ammonia production and nitrogen solubility. In general, nitrogen soluble in rumen fluid was a better indicator of ammonia formed than was

nitrogen soluble in diluted alkali or water. This was particularly evident in the in vivo experiments with purified proteins. Casein, being more than 80 percent soluble in rumen fluid, resulted in the highest ammonia level. Zein, which was practically insoluble in rumen fluid but highly soluble in dilute sodium hydroxide, produced only a slight increase in free ammonia. Soy protein, analyzed to be less than 10 percent soluble in rumen fluid, was higher than zein in ammonia formed. Regular soybean oil meal contained approximately twice as much soluble nitrogen as heated soybean oil meal in the rumen fluid solvent and, likewise produced a higher level of ammonia when administered alone. Perhaps under the influence of readily available carbohydrates contained in the mixed ration, ammonia was utilized more rapidly than it was formed, and no difference between regular and heated soybean oil meals was noted when they were administered in this manner.

The results of the in vitro cellulose digestion experiment paralled the results of in vitro ammonia production. Those proteins that were rapidly converted to ammonia were effective sources of nitrogen for cellulose digestion. Those that were not readily converted to ammonia were ineffective. These results suggest that a soluble nitrogen source is essential for optimum rumen microbial activity in vitro.

The in vitro results were not completely confirmed by the lamb feeding experiments. Heated soybean oil meal was of equal value to regular soybean oil meal in both the natural rations containing low quality

roughages and the semi-purified rations. Linseed meal was similar in feeding value to soybean oil meal. Corn gluten meal, being similar to heated soybean oil meal in nitrogen solubility, ammonia formation and in vitro cellulose digestion, was much inferior for promoting lamb growth.

Summary

The significance of soluble nitrogen of feed proteins was investigated. The soluble nitrogen content of several feeds and purified proteins was determined using three different solvents. Marked differences were noted between protein sources as well as within the same source in different solvents. Heat was shown to reduce nitrogen solubility. Rate of ammonia production from some of these feeds was measured. No definite relation was evident; however, nitrogen solubility in rumen fluid was generally more indicative of ammonia formed than solubility in dilute sodium hydroxide or distilled water. The influence of a number of feed proteins on in vitro cellulose digestion was found to be comparable to extent of microbial attack measured by ammonia formation. Reduction of soluble nitrogen in soybean oil meal by heat treating was of little significance in lamb rations. Corn gluten meal, low in soluble nitrogen, only slightly converted to ammonia and ineffective as a nitrogen source for in vitro cellulose digestion, was also inferior as a protein source in a semi-purified lamb ration.

Significance of the Water Soluble Fraction of Soybean Oil Meal

The occurrence of unidentified factors stimulatory to rumen function has been reviewed. Such factors appear to be most prevalent in high protein feeds and have been suggested by many to be the basis of protein quality for ruminant animals. Soybean oil meal has been identified as a highly potent source of so called "protein quality factors." There are reports, particularly from in vitro experiments, that characterize unidentified factors in soybean oil meal and in certain other feeds as being water soluble. The results of preceding phases of this work did not entirely support an idea that soluble nitrogen content of feeds was indicative of their protein feeding value. Information was desired concerning the actual value of the water soluble fraction of soybean oil meal in ruminant rations.

Preliminary studies were conducted in vitro to test the stimulatory activity of a water extract preparation and some characteristics of the active factors. A digestibility and nitrogen balance trial was conducted to validate the use of a semi-purified ration for studying the responses to unidentified factors. The water extract was prepared in sufficient quantities for feeding trials, and its effect on lamb performance determined.

Methods and results

Influence of soybean oil meal fractions on in vitro cellulose digestion by rumen microorganisms The in vitro artificial rumen has been used successfully in many studies of unidentified factors. The washed cell suspension technique, in particular, offers an advantage of studying requirements of rumen microorganisms in a strictly defined nutrient medium. This technique was used in the present experiment to study the effect on cellulose digestion of a water extract preparation from soybean oil meal. An attempt was also made to obtain knowledge of the character of the active factors present in this fraction. Several attempts to characterize the factors present in similar feed fractions have been reported; however, the present status of the properties of the active factors is rather unsettled.

The in vitro procedure has been previously described. The nutrient medium used in this trial was similar to the medium previously used, the contents of which were given in Table 4. The only change was that 1 gram of urea was added per liter to provide a nitrogen source.

The water soluble fraction used in this experiment was prepared by suspending finely ground soybean oil meal in warm water (80° C) and agitating for 30 minutes. A ratio of 1 part meal to 4.5 parts water on a weight basis was used. The liquid was separated by centrifuging in a high speed Servall centrifuge at 12,000 r.p.m. for 5 minutes. The resulting supernatant represented the water soluble fraction and has been

designated "extract." It contained an average of 7.8 percent dry matter and 37 percent crude protein on the dry matter basis.

The various procedures conducted in an attempt to characterize the active factors in the extract are described below. To a 100 ml. portion of the extract were added 200 ml. absolute ethanol previously chilled to 0° C. This extract-alcohol mixture was refrigerated at 5° C for 6 hours, followed by removing the precipitate by filtration. The precipitate was air dried and a sample analyzed for crude protein. An average total of 3.6 grams air-dried material containing 70 percent crude protein was obtained. This fraction was designated "alcohol ppt." The filtrate from this extract-alcohol mixture was evaporated to near dryness over a steam pot and the volume brought to 100 ml. with distilled water. This fraction is designated "alcohol filtrate."

To a second 100 ml. portion of the extract was added sufficient 0.1N HCl to bring the solution to pH 4.4. This acid solution was refrigerated at 5° C for 6 hours, and the formed precipitate removed by filtration. A total of 2.7 grams air-dry material was usually obtained containing approximately 78 percent crude protein. This fraction was designated "pH 4.4 ppt."

A 10 ml. portion of the extract was evaporated to dryness and ashed at 550° C for 2 hours. The ash was redissolved in 10 ml. distilled water.

Fifty ml. of the extract were placed in a separatory funnel and

25 ml. ether added. The funnel was stoppered and agitated, and after setting for 30 minutes, the aqueous phase was collected. This ether washing was repeated and the aqueous phase again collected. This fraction was designated "ether washed extract."

Each of these fractions was added to the artificial rumen tubes at levels equivalent to the original amount of soybean oil meal from which they were obtained. For example, when 1 gram of soybean oil meal was extracted with 4.5 ml. water, 0.45 ml. of the extract was equivalent to 100 mg. of the meal. The extract and fractions added in trial 1 and trial 2 were prepared at different times. The samples used in trials 3 and 4 were from a common preparation. The Tukey test as described by Snedecor (92) was used to determine a difference required for significance.

The results of this study are presented in Table 10. The water soluble fraction of soybean oil meal (extract) consistently stimulated a marked improvement in cellulose digestion. When two levels were compared, the response was essentially additive. The responses from the various fractions of the extract were less consistent.

The alcohol precipitate and alcohol filtrate particularly produced variable responses. In trial 1 alcohol filtrate was more active in stimulating cellulose digestion than the alcohol precipitate. The latter showed no activity and when the two were combined, they were only slightly more active than the filtrate alone. In trial 2 the precipitate and

Table 10. Influence of water extract of soybean oil meal and certain fractions thereof on *in vitro* cellulose digestion by rumen microorganisms

Treatment	Percent cellulose digested						
	Trial 1[a]	Trial 2[b]		Trial 3[c]		Trial 4[d]	
	100 mg. eqv.	75 mg. eqv.	150 mg. eqv.	75 mg. eqv.	150 mg. eqv.	75 mg. eqv.	150 mg. eqv.
Control (20 mg. urea)	31.3	27.0	27.0	11.2	11.2	26.7	26.7
SBOM extract	52.0	36.0	46.2	33.4	60.4	46.6	61.2
Alc. ppt. of extract	31.9	33.2	26.3	23.1	28.8	32.0	30.9
Alc. filtrate of extract	43.0	31.8	30.7	17.3	42.5	21.3	19.9
Alc. ppt. plus alc. filtrate	45.9	--	--	24.0	64.4	33.4	40.5
pH 4.4 ppt. of extract	48.4	37.3	36.2	--	--	--	--
Ash of extract	30.6	25.8	24.9	--	--	--	--
Ether washed extract	53.2	--	--	--	--	--	--
Urea-N	31.4	--	--	10.9	14.4	--	--
Soybean oil meal	--	37.3	43.0	--	--	--	--

[a]A difference (D) of 10.4 required for significance at P = .05 level for comparison of treatment means.

[b]D = 8.5.

[c]D = 5.6.

[d]D = 5.2.

filtrate contained about the same amount of activity, but neither gave much stimulation. The precipitate was inactive when added at the 150 mg. equivalent level. In trials 3 and 4 there appeared to be some activity in the precipitate; however, when the level was increased there was no increase in stimulation. In trial 3 the filtrate contained a sizable amount of activity when added at the 150 mg. equivalent level. The combined precipitate and filtrate at this level reached the stimulatory effect of the original extract. The filtrate did not increase cellulose digestion in trial 4 when added alone, but it did slightly improve digestion when added in combination with the precipitate.

The pH 4.4 precipitate fraction was active in each of the two trials it was compared. It contained essentially all of the active principle when added at 75 mg. and 100 mg. equivalent levels but only about half of the original at the 150 mg. equivalent level.

Ash of the extract did not affect cellulose digestion at any of the three levels added. Ether washing did not remove any of the activity from the extract in trial 1.

The responses from extract were apparently not due simply to available nitrogen, for in trials 1 and 3 added urea at levels equivalent to the nitrogen in the extract did not significantly benefit cellulose digestion. It should be emphasized that a supposedly adequate level of nitrogen was present in all of the tubes because 1 gram urea per liter was contained in the medium.

It appeared from trial 2 that most of the active principle in soybean oil meal was in the water soluble fraction. Additions of 75 and 150 mg. soybean oil meal were no more effective than the extract from equivalent amounts.

Digestibility and nitrogen balance trial with lambs to validate the use of a semi-purified ration to study effects of unidentified factors

Most studies of the effects of unidentified factors on growth have been conducted using purified rations containing purified proteins or urea as the nitrogen source. Feed consumption is often low on such rations, and as a result animal growth is curtailed. An ideal situation in such studies would be to feed a basal ration which would be readily consumed yet low in the factors being studied.

In most cases as the natural ingredients of lamb rations are increased, feed consumption likewise increases; however, most natural protein feeds contain unidentified factors. In the preceding lamb trial with semi-purified rations, the growth of lambs receiving corn gluten meal was much inferior to lambs receiving soybean oil meal. Apparently soybean oil meal contained factors that were either not present or not available in corn gluten meal. The feed consumption of the lambs on corn gluten meal was fairly adequate, although not of the unusually high level of the other treatments. Perhaps a semi-purified ration containing corn gluten meal as the nitrogen source would be sensitive to unidentified factor additions while being more palatable than highly

purified rations. Additional evidence on the comparative values of these rations was needed.

This experiment was initiated to compare corn gluten meal with soybean oil meal in digestibility and nitrogen balance. It was hoped that information obtained would indicate whether the preceding inferior growth on the corn gluten meal ration was due to poor digestibility and utilization of the protein or whether corn gluten meal contained insufficient or unavailable rumen stimulatory factors. A positive answer to the latter would suggest validation of the use of this ration to study growth promoting properties of unidentified factors.

Sixteen lambs with an average weight of 91 pounds were used in this digestibility and nitrogen balance trial. The lambs were randomly divided into two groups of eight and placed in individual pens for a ration adjustment period. The lambs in one group were individually fed a semi-purified ration containing corn gluten meal as the nitrogen source, while the lambs in the other group were individually fed a similar ration containing soybean oil meal as the nitrogen source. During this adjustment period of 14 days, the lambs were fed a constant amount (908 grams) of their respective rations daily. The lambs were then placed in steel stanchion type metabolism stalls for successive 4-day preliminary and 10-day collection periods. Daily individual allowances of the rations were 908 grams which were fed in equal portions twice daily. Water was available at all times. The composition of the rations fed was

identical to rations 1 and 4 shown previously in Table 8.

The feces were collected daily and dried for 24 hours in a forced air oven at approximately 70° C. The total dried fecal collection was allowed to moisture equilibrate with the air. The dried fecal material was then mixed, sampled and ground for analysis. The procedure of Crampton and Maynard (33) was used for determining cellulose, and accepted procedures of the Association of Official Agricultural Chemists (6) were used for other determinations.

The urine was filtered through glass wool and collected in glass jars containing sufficient HCl to maintain acidity. Daily urine collections were diluted with water to a constant volume, and 5 percent aliquot samples taken. The urine samples were stored under refrigeration until analyzed.

The nutrient composition of the rations as determined is given in Table 11. The two rations were practically equal in dry matter, organic matter, protein, cellulose and ash. Digestibility and nitrogen balance data are presented in Table 12. These data were analyzed and tested for significance by the F test according to Snedecor (92). The statistical analyses are summarized in Table 26 of the Appendix.

There was a marked difference in cellulose digestibility between the two rations. The corn gluten meal fed lambs digested only 50.21 percent of the total ration cellulose, and the soybean oil meal fed lambs digested 61.19 percent of the ration cellulose. This difference was

Table 11. Average percentage composition of rations fed to lambs in digestion and nitrogen balance trials (air dry basis)

	Ration	
	1 Corn gluten meal	2 Soybean oil meal
Dry matter	92.6	92.0
Organic matter	87.2	86.1
Protein	8.6	8.5
Cellulose	29.8	29.9
Ash	5.4	5.9

significant at the 0.05 level of probability and approached significance at the 0.01 level.

There was essentially no difference in protein digestibility or nitrogen balance between the two rations. The difference in cellulose digestibility was reflected in the difference in organic matter digestibility.

These results were interpreted as indicating that corn gluten meal is equal to soybean oil meal in providing protein to the lamb; but corn gluten meal is inferior in stimulating rumen function. Thus, the use of the corn gluten meal ration to study the effect of rumen stimulatory factors was validated.

Table 12. Digestibility and nitrogen balance data obtained with lambs fed corn gluten meal or soybean oil meal as protein source in semi-purified ration

	Ration	
	1	2
	Corn gluten meal	Soybean oil meal
Number of lambs	8	8
Dry matter intake (gm.)	841.0	835.2
Digestibility (%)		
Organic matter	71.08	75.04
Protein	67.49	65.62
Cellulose	50.21	61.19
Daily nitrogen balance (gm.)		
Nitrogen intake	12.52	12.35
Nitrogen in feces	4.07	4.25
Nitrogen in urine	4.34	4.17
Nitrogen retained	4.12	3.91
Nitrogen retained as percent intake	32.85	31.66

Effect on lamb performance of water soluble fraction of soybean oil meal Numerous in vitro experiments have been reviewed which indicate that the water soluble fraction of several protein feeds is stimulatory to rumen function. Soybean oil meal has been commonly listed as one of the more active sources. The preceding in vitro experiments in this study had confirmed that the water soluble fraction of soybean oil meal was active in stimulating cellulose digestion by rumen microorganisms.

A series of lamb feeding trials was initiated to study further the stimulatory properties of this protein feed fraction. Since the results of a preceding lamb growth trial and digestibility trial suggested that corn gluten meal was inferior to soybean oil meal for promoting growth and in vivo cellulose digestion but was equal in protein digestibility and nitrogen balance, this was interpreted that corn gluten meal inadequately provided stimulatory factors which were seemingly present in soybean oil meal. A semi-purified ration containing corn gluten meal was thus chosen as the basal ration to study the effect of soybean extract additions.

The water soluble fraction of soybean oil meal was prepared for these feeding trials in a similar manner as described in the in vitro studies, only on a larger scale. Soybean oil meal was suspended in hot water at 80° C and stirred with a mechanical stirrer for 30 minutes. A ratio of 1 part meal to 4.5 parts water on a weight basis was normally used. The liquid was separated by straining the suspension through several grades of screen wire. A device similar to a lard press was used. Only about half of the original liquid volume was recovered as extract. In most cases the liquid extract was poured into shallow pans and placed in a forced air drying oven. The liquid was evaporated at an oven temperature of 80° C. Approximately 36 to 48 hours were required in the drying oven to remove all of the liquid. A small amount of finely ground corn cobs was added to each pan to hasten drying and to facilitate removal of the dried extract. The exact amount added to each pan was

recorded and adjustments were made in recovery calculations as well as in feeding levels. Upon removal from the pans, the dried extract was weighed and ground preparatory to feeding.

The dried extract contained an average of 39 percent crude protein. Approximately 70 grams of dried extract were obtained from each pound of soybean oil meal extracted.

The first trial in this series involved the individual feeding of twenty-four lambs. Six lambs were randomly assigned to each of the four treatments. These lambs had been used in a preceding experiment, so they were fed the respective rations in individual pens for a 10 day standardization period. During this standardization period, all lambs were fed a constant amount of 2.5 pounds daily. The average weight of the lambs at the beginning of the experimental period was 92 pounds.

The composition of the rations fed in this trial was identical to rations 1 and 4 given previously in Table 8. Lambs on treatments 1 and 2 were fed the ration containing soybean oil meal as the protein source, and lambs on treatments 3 and 4 received the ration containing corn gluten meal as the protein source. The animals on treatments 2 and 4 each received 20 grams of dried soybean oil meal extract daily in addition to the respective basal rations.

Two levels of soybean oil meal extract additions to the basal ration containing corn gluten meal were studied in the second lamb feeding trial in this series. A treatment was included as a positive control in

which soybean oil meal was fed as the protein source. The semi-purified basal rations were of the same composition as previously stated. Twenty-four lambs with an average initial weight of 74 pounds were used in this trial. They were vaccinated for enterotoxemia and sore mouth upon arrival and were group fed the corn gluten meal ration for 7 days. Lamb assignments to the four treatments were random and the lambs were placed in individual pens with free access to feed and water throughout the 55 day experimental period. Dried extract supplementations were made at the rate of 20 and 40 grams per lamb daily in treatments 2 and 3, respectively.

In the first two trials of this series the influence of dried extract was determined when it was added to a basal ration. This represented a slight increase in total nitrogen content of the supplemented rations. Although this supplementation did not amount to a sizable increase in ration nitrogen, perhaps it was influential since the basal ration contained a marginal protein content of approximately 8 percent. The primary objective of the third trial in this series was to determine the effect of soybean oil meal extract when fed in equal nitrogenous rations. An additional 2 pounds of corn gluten meal per hundred were added to the control ration to bring the protein level up to extract supplemented rations. A second objective of this trial was to determine the effect of drying the extract on its activity. Liquid extract was added to the ration fed to the lambs on treatment 3 at the rate of 0.5 pound per pound of basal ration.

This level of liquid extract provided approximately the same amount of dry matter as 16 grams of dried extract per pound of ration fed in treatment 2. The soybean oil meal ration was again fed as the positive control. Water was added to the rations of treatments 1, 2 and 4 to equal the amount of moisture added as liquid extract in treatment 3.

The lambs used in this experiment had been used in a previous experiment and were fed the respective rations for a 10 day standardization period before the 26 day experimental period was begun. They were placed in individual feeding crates for two 3-hour periods daily throughout the experiment. Free access to water and block salt was allowed while not in the feeding crates.

The average daily gain and feed consumption of the lambs in the first trial (Table 13) were exceptionally good. It will be recalled that similarly good performance was reported when this same group of lambs was used in the semi-purified ration study reported in the preceding section of this work. Soybean oil meal was superior to corn gluten meal as can be noted by both increased gain and feed consumption. When corn gluten meal was supplemented with 20 grams dried soybean extract daily, it was comparable to soybean oil meal. Supplementation of soybean oil meal with the extract only slightly improved rate of gain. Although due to wide individual variation these differences in gain were not significant (Table 27 of Appendix), they do indicate that the water soluble fraction of soybean oil meal contains factors responsible for superior performance

Table 13. Performance data of lambs fed soybean oil meal or corn gluten meal supplemented with water soluble fraction of soybean oil meal

Treatment	30 day ave. daily gain 6 lambs (lbs.)	Ave. daily feed consumption (lbs.)
1. Soybean oil meal (SBOM)	.62	4.39
2. SBOM plus 20 gm. dried extract[a]	.68	4.19
3. Corn gluten meal (CGM)	.54	3.75
4. CGM plus 20 gm. dried extract[a]	.68	3.83

[a]Water soluble fraction of soybean oil meal, dried and fed daily to lambs individually.

of soybean oil meal over corn gluten meal.

The results of the second lamb trial in this series are summarized in Table 14. In accordance with previous results, corn gluten meal was inferior to soybean oil meal as a protein source in the semi-purified ration. Corn gluten meal failed to produce average positive gain. The additions of the water soluble fraction of soybean oil meal to corn gluten meal increased both gain and feed consumption. The responses from the two levels of extract were essentially additive. Perhaps due to the much lower performance on the corn gluten meal basal ration, the extract supplemented lambs did not approach the performance of the soybean oil meal fed lambs in this trial. Statistical analyses of these data are

Table 14. Effect on lamb performance of feeding water soluble fraction of soybean oil meal

Treatment	55 day ave. daily gain 6 lambs (lbs.)	Ave. daily feed consumption (lbs.)
1. Corn gluten meal (CGM)	-.02	1.71
2. CGM plus 20 gm. dried extract[a]	.11	1.90
3. CGM plus 40 gm. dried extract[a]	.20	2.20
4. Soybean oil meal	.45	3.06

[a]Water soluble fraction of soybean oil meal, dried and fed daily to lambs individually.

summarized in Table 28 of the Appendix.

In the third lamb trial of this series (Table 15) lambs fed soybean oil meal again gained at a significantly more rapid rate than corn gluten meal fed lambs. This superior gain was produced without a large difference in feed consumption. The value of corn gluten meal approached the value of soybean oil meal when the former was supplemented with the water soluble fraction of the latter. Dried extract was almost equally effective as liquid extract for improving performance of lambs fed corn gluten meal. These results are statistically analyzed in Table 29 of the Appendix.

Table 15. Effect on lamb performance of ration additions of soybean oil meal water extract in dried and liquid forms

Treatment[a]	Number of lambs	26 day ave. daily gain (lbs.)	Ave. daily feed consumption (lbs.)[b]
1. Corn gluten meal (CGM)	7	.20	1.73
2. CGM plus dried extract	8	.38	2.04
3. CGM plus liquid extract	8	.42	2.02
4. Soybean oil meal	7	.47	1.94

[a]Rations fed in treatments 1, 2 and 4 contained 50% added water, equivalent to moisture in liquid extract.

[b]Values adjusted to 92 percent dry matter.

Discussion

The stimulatory effect of the water soluble fraction of soybean oil meal on *in vitro* cellulose digestion was successfully repeated with three separate preparations in four artificial rumen trials. Several workers have reported similar results with water soluble fractions of other feeds (17, 23, 46, 59, 88, 93).

Although this active fraction was prepared from a high protein feed, its stimulatory properties cannot be accounted for on the basis of nitrogen *per se*. Concentration of unidentified factors was evident.

The attempt to characterize the unidentified factors in soybean oil

meal was not completely successful. The effects of the alcohol fractions (alcohol precipitate and alcohol filtrate) were very inconsistent, and this inconsistency prevents any conclusions concerning these fractions. The lack of stimulation when these two fractions were combined certainly cannot be explained. One might interpret these results as indicating that active factors were destroyed in the alcohol fractionation procedure; however, it is possible that these fractions may have been contaminated with traces of alcohol residue which limited their effectiveness. Other workers have reported that the active factors present in similar feed preparations were soluble in dilute alcohol but insoluble in concentrated alcohol (17, 88).

The response from the extract precipitate of pH 4.4 was rather consistent and suggests that the protein material in soybean oil meal can be concentrated considerably without loss of stimulatory activity. Ruf (88), however, did not remove the activity from a solution of a similar preparation by other protein precipitation procedures. The amino acid composition of the protein of a similar preparation has been reported by Van Etten et al. (96).

Ash of the extract did not affect cellulose digestion. This is in agreement with Baker et al. (7) and Ruf (88). These workers concluded from their studies that unidentified factors contained in the water soluble fraction of certain protein feeds were organic in nature.

The marked difference in cellulose digested by lambs fed corn gluten

meal and lambs fed soybean oil meal indicated that corn gluten meal was inferior for promoting rumen function. The similarity between these two protein feeds in nitrogen balance did not suggest a difference in utilization of protein. Apparently corn gluten meal was inadequate in rumen stimulatory factors; or if such factors were contained, they were unavailable.

Results of the lamb feeding trials confirmed the in vitro results as well as in vitro results reported by other workers. The water soluble fraction of soybean oil meal was beneficial to lamb performance when added to a low quality protein ration. The activity of this fraction does not appear to be decreased by drying at a high temperature.

Summary

The significance of the water soluble fraction of soybean oil meal in ruminant nutrition was investigated. This fraction stimulated in vitro cellulose digestion by rumen microorganisms. This fraction also improved the performance of lambs fed a semi-purified ration containing corn gluten meal as the major protein source.

The water soluble fraction of soybean oil meal appeared to be of major significance, and factors present in this fraction are perhaps responsible for the high quality of this protein feed.

Significance of Available Nitrogen and Amino Acid Balance in a Low Quality Protein Feed

Corn gluten meal definitely appeared to be a low quality protein feed for ruminants from preceding experimental findings. It was evident that corn gluten meal was inferior to soybean oil meal as a protein source both in vitro and in semi-purified lamb rations. It was also established that corn gluten meal could be improved by supplementation with the water soluble fraction of soybean oil meal. The "protein quality factors" which appeared to be inadequate in corn gluten meal were apparently present in soybean oil meal and were concentrated in the water soluble fraction.

Zein, which comprises a large portion of corn gluten meal protein, has been reported to be poorly attacked by rumen microorganisms. This was supported by ammonia formation data presented in the first section of this work. One might reason that corn gluten meal is not a readily available source of nitrogen. Corn protein is known to be low in the amino acid lysine. Although lysine has been shown to be synthesized in the rumen, it may be a limiting factor if corn protein is not extensively converted to microbial protein.

Information was desired concerning the significance of readily available nitrogen and amino acid balance in a ruminant ration. Both in vitro experiments and lamb feeding experiments were conducted to obtain this information. It was hoped that such knowledge would help to illucidate the "quality factors" present in soybean oil meal and

supposedly inadequate in corn gluten meal.

Methods and results

Effect of urea, lysine and methionine on in vitro cellulose digestion with corn gluten meal furnishing the major source of nitrogen The washed cell suspension artificial rumen technique of Cheng et al. (31) was used in this study. The modifications in the original procedure and the composition of the nutrient medium have previously been described (Table 4).

Analytical grade urea and technically pure L-lysine HCl and DL-methionine were used. Fresh solutions of these compounds were made for each experiment. Corn gluten meal was very finely ground, and immediately before each experiment it was suspended in water by using a magnetic stirrer and pipetted into the digestion tubes. Each treatment was replicated in three tubes during each trial. Preparations of the soybean oil meal extract have been previously described.

Urea and lysine were selected as treatments for reasons stated in the introduction to this section. Methionine was chosen because it has been linked with rumen function by several workers. Although corn gluten meal is supposedly adequate in methionine, if the protein is not attacked by the bacteria this source would not be available. The Tukey test described by Snedecor (92) was used to determine a difference required for significance in each trial.

The effects of urea and lysine additions on in vitro cellulose digestion by rumen microorganisms are presented in Table 16. Similar to preceding results, corn gluten meal was not an effective source of nitrogen for in vitro microbial activity. The rate of cellulose digestion, when corn gluten meal was added was no better than when no nitrogen was added. When corn gluten meal was supplemented with urea, a very dramatic increase in digestion resulted. This increase far exceeded the increase from urea additions alone. Lysine tended to slightly increase the value of corn gluten meal, although not significantly. The 2 milligram level of lysine was essentially equal to the 75 milligram equivalent of soybean oil meal extract. The higher level of extract significantly increased the value of corn gluten meal.

In the second experiment (Table 17) lysine and methionine additions were made to urea and corn gluten meal. Various combinations of these substances were also added. Methionine slightly improved urea and significantly improved corn gluten meal. Lysine had little effect on either urea or corn gluten meal. When methionine and lysine were combined, their effect was essentially equal to methionine alone. As in the first experiment, urea markedly improved corn gluten meal. The amino acid additions were without effect when added to the urea-corn gluten meal combination.

The results of the third experiment (Table 18) further confirmed that corn gluten meal alone was totally ineffective as a nitrogen source

Table 16. Comparative responses in cellulose digestion in vitro by rumen microorganisms to additions of urea, lysine and soybean oil meal extract to corn gluten meal

Treatment Additions/20 ml.	Percent cellulose digested[a]
No N	33.6
8 mg. urea	47.1
20 mg. urea	46.9
50 mg. corn gluten meal (CGM)	32.5
50 mg. CGM plus 4 mg. urea	62.1
50 mg. CGM plus 8 mg. urea	64.7
50 mg. CGM plus 1 mg. lysine	35.2
50 mg. CGM plus 2 mg. lysine	37.3
50 mg. CGM plus 75 mg. eqv. SBOM extract	38.9
50 mg. CGM plus 150 mg. eqv. SBOM extract	42.3

[a]A difference of 7.2 required for significance at P = .05 level for comparison of treatment means.

for in vitro cellulose digestion. The superiority of soybean oil meal was again illustrated. In trial 1 both corn gluten meal and soybean oil meal were improved by additions of urea; however, the former improvement was more dramatic. Corn gluten meal and urea combined exceeded soybean oil meal alone in promoting cellulose digestion and approached the

Table 17. Comparative responses in cellulose digestion in vitro by rumen microorganisms to urea, corn gluten meal, lysine and methionine

Treatment Additions/20 ml.	Percent cellulose digested[a]
20 mg. urea	39.2
20 mg. urea plus 2 mg. lysine	38.6
20 mg. urea plus 4 mg. methionine	42.8
20 mg. urea plus 2 mg. lysine plus 4 mg. methionine	43.2
50 mg. corn gluten meal (CGM)	28.0
50 mg. CGM plus 2 mg. lysine	31.1
50 mg. CGM plus 4 mg. methionine	36.7
50 mg. CGM plus 2 mg. lysine plus 4 mg. methionine	37.1
20 mg. urea plus 50 mg. CGM	59.2
20 mg. urea plus 50 mg. CGM plus 2 mg. lysine	56.5
20 mg. urea plus 50 mg. CGM plus 4 mg. methionine	60.0
20 mg. urea plus 50 mg. CGM plus 2 mg. lysine plus 4 mg. methionine	58.1

[a]A difference of 6.4 required for significance at P = .05 level for comparison of treatment means.

soybean oil meal-urea combination. In the second trial soybean oil meal was not improved by the urea addition, and cellulose digestion with the corn gluten meal-urea combination exceeded that with soybean oil meal. Soybean extract was more active in the presence of urea in both trials.

Influence on lamb performance of available nitrogen and amino acid additions to semi-purified rations The above reported results indicated a dramatic response in in vitro cellulose digestion when corn gluten meal was supplemented with urea. Lysine and methionine additions resulted in small stimulations. This suggested, along with ammonia formation data presented in a preceding section, that perhaps available nitrogen was a limiting factor in corn gluten meal. Two lamb feeding trials were conducted to further determine the significance of available nitrogen and amino acid adequacies.

The compositions of the rations fed on the respective treatments in the first trial are given in Table 19. Various treatment additions were made to the corn gluten meal basal ration. A portion of corn gluten meal was omitted from those rations to which an addition was made so that the rations would be isonitrogenous. The preparation of soybean oil meal extract included in ration 2 has been previously described. The soy hydrolysate included in ration 3 was an enzymatic preparation obtained from National Biochemicals Corporation, Cleveland, Ohio. It contained 57 percent crude protein by analysis and was certified by the manufacturer to contain 3.3 percent amino nitrogen. Its nitrogen content was 100 percent soluble in water when determined by the solubility technique previously described. The level of soy hydrolysate included in ration 3 as well as the level of urea included in ration 4 were equivalent on a nitrogen basis to the level of extract included in ration 2. All of these rations

Table 18. Comparative responses in cellulose digestion in vitro by rumen microorganisms to urea additions to corn gluten meal, soybean oil meal and soybean oil meal extract

Treatment Additions/20 ml.	Percent cellulose digested	
	Trial 1[a]	Trial 2[b]
No N	19.6	20.5
8 mg. urea	34.3	37.5
50 mg. corn gluten meal (CGM)	20.0	22.9
50 mg. soybean oil meal (SBOM)	43.2	58.2
50 mg. eqv. SBOM extract	23.9	29.7
150 mg. eqv. SBOM extract	--	40.0
8 mg. urea plus 50 mg. CGM	63.3	65.4
8 mg. urea plus 50 mg. SBOM	70.3	57.7
8 mg. urea plus 50 mg. eqv. SBOM extract	54.3	54.4
8 mg. urea plus 150 mg. eqv. SBOM extract	--	56.4

[a]A difference (D) of 4.9 required for significance at P = .05 level for comparison of treatment means.

[b]D = 6.6

were formulated to contain 8 percent crude protein.

Fifty-six crossbred lambs were individually fed in this first trial. They were vaccinated for enterotoxemia and sore mouth and drenched with phenothiazine prior to the start of the experiment. The lambs were group fed the corn gluten meal basal ration (ration 1) for a 10-day adjustment period, after which time they were randomly alloted to the 7 treatments. Because of respiratory infections in many of the lambs probably due to extreme weather conditions, the experimental period was not begun at time of allotment. They were fed the respective rations for

Table 19. Composition of semi-purified rations fed to lambs in trial 1

Ration ingredient	Ration						
	1	2	3	4	5	6	7
Corn cobs	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Cellulose[a]	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Corn dextrose	14.3	14.1	14.5	15.2	14.3	21.4	14.4
Corn starch	13.2	13.1	13.5	14.2	13.3	20.4	13.3
Corn oil	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Mineral mixture[b]	6.3	6.3	6.3	6.3	6.3	6.3	6.3
Syrup[c]	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin mixture[d]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Corn gluten meal	17.2	14.9	14.9	14.9	17.0	--	--
Soybean oil meal	--	--	--	--	--	--	17.0
SBOM extract	--	2.6	--	--	--	--	--
SBOM hydrol.	--	--	1.8	--	--	--	--
Urea[e]	--	--	--	0.4	--	2.9	--
L-lysine HCl	--	--	--	--	0.1	--	--

[a]Solka-floc, purified wood cellulose.

[b]Composition of mineral mixture given in Table 22 of Appendix.

[c]Composed of 3.75 pounds sucrose and 1.25 pounds water.

[d]Composition of vitamin mixture given in Table 23 of Appendix.

[e]Feed grade, 262 protein equivalent.

an additional 12-day preliminary period until most of the infection symptoms were no longer evident. The lambs started the experiment weighing an average of 54 pounds. During the 41-day experimental period that followed, half of the lambs (four from each treatment) were kept in individual pens with access to feed and water at all times. The other half were fed in individual feeding crates for two 3-hour periods daily. While not in the feeding crates, the latter lambs were placed in a holding lot with free access to water.

The performance of the lambs on this trial (Table 20) were generally poor with wide variations within each treatment (Table 30 in the Appendix). Feed consumption was unusually low, and considerable coprophagy was observed during the experimental period. The equal performance on corn gluten meal and corn gluten meal supplemented with soybean extract in this trial are not in keeping with superior results obtained with extract supplementation in previous lamb trials. Extract feeding did not improve either feed consumption or rate of gain in this trial. Likewise, soy hydrolysate and urea supplementations did not improve lamb performance. Both tended to decrease rate of gain and feed consumption slightly. Lysine significantly reduced rate of gain. In accord with previous results soybean oil meal fed lambs did gain at a significantly more rapid rate than corn gluten meal fed lambs, and in this trial on little increase in feed consumption. Urea as the major source of nitrogen in this ration was much inferior to both corn gluten meal and soybean oil meal.

Table 20. Effect on lamb performance of ration supplementation with several nitrogenous substances

Treatment	41 day ave. daily gain of 8 lambs (lbs.)	Ave. daily feed consumption (lbs.)
1. Corn gluten meal (CGM)	.14	1.69
2. CGM and SBOM extract	.16	1.70
3. CGM and soy hydrolysate	.06	1.55
4. CGM and urea	.05	1.49
5. CGM and lysine	-.02	1.43
6. Urea	-.01	1.32
7. Soybean oil meal	.35	1.88

In the above lamb trial it appeared that feed consumption decreased as ration content of natural feeds was decreased. To test further the significance of available nitrogen in a corn gluten meal ration a second trial was initiated in which various additions were made above the contents in the basal ration. Knowledge of the effect of various additions to rations containing equal amounts of natural feed ingredients was desired.

The following rations were fed in this second trial. Ration 1 was identical to the corn gluten meal basal ration listed in Table 19. To ration 1 was added 1.04 percent crystalline urea for ration 2; 0.96 percent urea, 0.1 percent L-lysine HCl and 0.2 percent DL-methionine

for ration 3; and 0.70 percent urea and 2.7 percent dried soybean oil meal water extract for ration 4. Ration 5 was identical to the soybean oil meal ration listed in Table 19. Rations 1 and 5 were calculated to contain 8 percent crude protein, and rations 2, 3 and 4 were calculated to contain 11 percent crude protein.

Although protein was increased, comparisons could be made of the effect of the specific additions. Urea additions would indicate whether available nitrogen was limiting the performance of lambs fed corn gluten meal. By combining urea, lysine and methionine it could be determined whether these amino acids were limiting in addition to available nitrogen. By combining urea and extract a measurement could be made as to whether this fraction from soybean oil meal contained stimulatory factors other than available nitrogen that were limiting in corn gluten meal.

Thirty lambs of California origin were used in this feeding trial. They were vaccinated for enterotoxemia and sore mouth and group-fed the soybean oil meal ration (ration 5) for 7 days prior to the beginning of the experiment. Six lambs were randomly assigned to each of the 5 experimental rations. They were fed in individual feeding crates for two 3-hour periods daily during the 42 day experimental period. Block salt and water were provided while not in the feeding crates.

The results of this trial are presented in Table 21, and Table 31 in the Appendix summarizes the statistical analyses. Urea additions to corn gluten meal markedly increased lamb gains. The addition of lysine

Table 21. Effect on lamb performance of ration additions of readily available nitrogen and amino acids

Treatment	42 day ave. daily gain of 6 lambs (lbs.)	Ave. daily feed consumption (lbs.)
1. 8% protein corn gluten meal (CGM)	.13	2.15
2. 11% protein CGM and urea	.38	2.75
3. 11% protein CGM, urea, lysine and methionine	.36	2.72
4. 11% protein CGM, urea and extract	.35	2.49
5. 8% protein soybean oil meal	.27	2.48

and methionine to the corn gluten meal-urea combination did not affect lamb performance. The addition of soybean oil meal extract did not improve gain over the corn gluten meal-urea combination, but a similar gain was obtained on a lower feed intake. Soybean oil meal was superior to corn gluten meal; however, soybean oil meal fed at an 8 percent protein level was not equal to corn gluten meal-urea fed at a 11 percent protein level.

Discussion

Readily available nitrogen definitely appeared to be a major significance in ruminant rations. Urea alone was an effective source of

nitrogen for in vitro cellulose digestion; corn gluten meal alone was ineffective; but when the two were combined, a very dramatic increase in cellulose digestion resulted. This complementary effect of a readily available nitrogen source and a natural protein feed has been previously indicated by Belasco (11) and Burroughs et al. (26).

A slight stimulation in in vitro cellulose digestion was observed in these experiments when lysine and methionine were added to urea or corn gluten meal. Trenkle (93) has reported improvements in cellulose digestion with methionine additions to urea in the artificial rumen. The slight stimulation from these amino acids when added to corn gluten meal may well have been a result of added available nitrogen. This is suggested by the lack of stimulation when lysine and methionine were added in combination with urea and corn gluten meal. The marked response in cellulose digestion with additions of soybean oil meal extract in the presence of urea nitrogen certainly suggests that there are stimulatory factors contained in addition to available nitrogen. This latter suggestion is also supported by the fact that soybean oil meal was more effective in supporting cellulose digestion than a comparable level of urea nitrogen.

The first lamb trial was designed with the objective of measuring the significance of several feed protein fractions: the significance of a readily available source of nitrogen (urea); the significance of soluble fragments of soy protein (hydrolysate); the significance of lysine in which

corn gluten meal is deficient and soybean oil meal adequate. The complete urea basal ration was included as a measure of possible inhibitory factors which may be present in corn gluten meal rather than an inadequacy of essential factors. This trial was almost completely unsuccessful in attaining the above objectives. The fact that small additions of urea, hydrolysate and lysine reduced lamb growth certainly cannot be explained on the basis of previous results. The lack of response from the addition of soybean extract was equally perplexing. Numerous in vitro experiments and three previous lamb trials in which the same basal ration was fed had indicated marked stimulations with extract supplementation. Although the extract used in this trial was prepared from soybean oil meal of a different origin than in the previous experiments, the soybean oil meal was apparently high quality. This was indicated by the excellent gain of the lambs fed soybean oil meal as the protein feed.

As was previously stated, the lambs in the above trial were extremely light weight feeders; and many required treatment for respiratory infections prior to the onset of the experiment. Perhaps this was responsible for the poor performance of many of the lambs and the wide individual variation. Physiological conditions may have been limiting to a greater extent than nutritional factors. The procedure followed in this trial was to omit corn gluten meal in proportion to the various additions made. Although rations were kept isonitrogenous by this procedure, the natural feed content of the supplemented rations was reduced.

As the natural feed content decreased feed consumption likewise was observed to decrease.

The various treatment additions in the second trial were made above the natural feed content at the expense of increasing ration nitrogen. Increasing ration protein equivalent 3 percentage units by adding urea to corn gluten meal markedly improved gain and feed consumption. This was in agreement with in vitro results. The lack of response from lysine and methionine above the response from urea suggested that these amino acids were not limiting in this ration. Similarly, the lack of response from soybean oil meal extract above urea indicates that unidentified factors supposedly contained in the extract were not limiting. These results thus suggest that the major limiting factor in corn gluten meal was readily available nitrogen.

Summary

The significance of possible inadequacies of corn gluten meal was investigated. Additions of readily available nitrogen sources and amino acids were made to corn gluten meal, and the effects of these additions were measured by in vitro microbial experiments and lamb feeding experiments. In vitro cellulose was favorably influenced by additions of urea when corn gluten meal provided the major source of nitrogen in the culture medium. The complementary effect of urea and corn gluten meal combined could not be accounted for solely on the basis of total

nitrogen. Additions of lysine and methionine to the culture medium slightly improved cellulose digestion when either urea or corn gluten meal furnished the major portion of nitrogen; however, these amino acids did not affect microbial activity when urea and corn gluten meal were combined to furnish the nitrogen.

The first lamb feeding trial was unsuccessful in confirming the in vitro results. Lamb performance was poor with wide variations. Another lamb feeding trial was conducted which successfully confirmed the in vitro results. Adding 3 percent crude protein equivalent in the form of urea to corn gluten meal markedly improved lamb performance. Lysine and methionine and soybean oil meal extract were ineffective in improving lamb gains when added to the urea-corn gluten meal combination.

Readily available nitrogen was suggested as being a major limiting factor in corn gluten meal; nevertheless, the presence of other factors in protein feeds was indicated.

GENERAL DISCUSSION

Two fractions of high quality protein feeds were established as being of major significance for rumen function; the water soluble portion and the readily available nitrogen content. Corn protein in the form of corn gluten meal was established as being low quality protein, and soybean oil meal was established as containing high quality protein.

The merits of the water soluble fraction of a high quality protein feed were illustrated by studying the effects of this fraction of soybean oil meal. A preparation of the water soluble portion of soybean oil meal was shown to consistently improve in vitro cellulose digestion by rumen microorganisms when included in a medium containing all known required nutrients. A similar preparation improved lamb performance in three of four feeding trials when incorporated in a marginal protein semi-purified ration likewise containing all known required nutrients.

The significance of readily available nitrogen was suggested when additions of urea were made to slowly available corn protein. For in vitro cellulose digestion by rumen microorganisms corn gluten meal and urea were much superior to corn gluten meal alone and somewhat superior to urea alone. Similarly, lamb performance was markedly improved by urea additions to a corn gluten meal ration.

Corn gluten meal was inferior to soybean oil meal in all experiments in which they were compared. Corn protein was shown to be of low

nitrogen solubility in rumen fluid and water, slowly decomposed by rumen microorganisms as measured by rate of ammonia production, ineffective as a nitrogen source for *in vitro* cellulose digestion by rumen microorganisms, inferior in promoting ration cellulose digestion *in vivo* by lambs, and not equal to soybean oil meal in semi-purified rations for lamb performance.

It was apparent from this work that readily available nitrogen is vital for rumen function. The optimum amount of the total ration nitrogen present as a readily available source was not investigated, but a critical ratio of readily available nitrogen to total nitrogen may well prove to be of great significance. The importance of such a ratio is suggested by present knowledge of rumen function. Rumen microorganisms very rapidly ferment soluble carbohydrates and more slowly degrade cellulose (4, 82). Nitrogen is required for both processes (11, 24, 26), and both processes provide energy for assimilation of nitrogen into microbial protein (5, 13, 77). Therefore, it seems reasonable to assume that adequate readily available nitrogen is required early in the fermentation process to utilize the energy of rapidly fermented carbohydrates.

If readily available nitrogen is present in excess of the amount needed for protein synthesis, it is likely to be lost by ruminal absorption as ammonia. On the other hand, less rapidly attacked nitrogen sources would not be lost by absorption and would provide nitrogen for the

utilization of energy from cellulose. One might postulate that more readily available nitrogen would be required in a high starch ration and more slowly available nitrogen advantageous in a high fiber ration. This is suggested by reported observations that urea can be utilized more efficiently in high grain rations and less efficiently in high roughage rations (14, 37, 87). Nevertheless, in vitro rumen experiments and the lamb digestion trial conducted in this study suggest that some readily available nitrogen is required to maintain an active fermentation process needed to extensively degrade cellulose.

To study further the significance of readily available nitrogen will require accurate techniques for measuring this property of protein feeds. Solubility of protein nitrogen has been suggested as an indication of readily available nitrogen; however, studies of the significance of soluble nitrogen reported in this work do not indicate that there is a definite relation. General trends were noted, and nitrogen soluble in rumen fluid was found to be somewhat related to rate of ammonia production and effectiveness of supporting in vitro cellulose digestion. All proteins, with the exception of purified soy protein, that contained more than 18 percent nitrogen soluble in rumen fluid were superior to those which contained less than 18 percent soluble nitrogen. Perhaps other solvents can be found to be better indicators of available nitrogen.

The insoluble nature of corn protein and the seemingly sluggish attack by rumen microorganisms reported in this work is well supported

by other experimental findings (2, 3, 68, 69). Similarly, others have reported corn proteins to be less than optimum as protein sources in ruminant rations under rather strictly defined conditions (50, 51, 94, 95).

Johnson et al. (54) have suggested that proteins which are not converted to microbial protein in the rumen and become available beyond the rumen should have the same biological value for ruminants as for nonruminants. Corn protein is deficient in lysine and has a low biological value for nonruminants. Corn gluten meal protein was found to be equally as digestible as soybean oil meal protein. Apparently, corn protein is readily digested in the abomasum and small intestines. If insufficient lysine were not synthesized in the rumen, optimum tissue growth would not proceed, and this may be partially the reason why corn gluten meal was inferior to soybean oil meal.

The nitrogen balance data did not support the idea that amino acids were the limiting factor in corn gluten meal. The results of this trial showed essentially no difference in nitrogen balance between corn gluten meal fed lambs and soybean oil meal fed lambs. Nevertheless, this observation of equal nitrogen balance should not be interpreted hastily. It was illustrated early in this work that ammonia was rapidly produced from soybean oil meal and only slowly produced from corn gluten meal when they were incubated with rumen liquor. Proven experimental findings have established that excess ammonia can be absorbed from the rumen and excreted as urea. Although no observations were made on ammonia

produced when these complete rations were ingested, the possibility exists that more of the nitrogen of soybean oil meal was absorbed as ammonia and excreted unused in the urine. If this was the case, then the soybean oil meal fed lambs could have actually utilized more of the ration nitrogen which was absorbed posterior to the rumen without showing a difference in nitrogen balance.

Several workers have recently reported improved performance of ruminant animals with lysine supplementation of high protein rations (10, 45, 84). The value of corn gluten meal was not seemingly improved in these studies by additions of lysine in a marginal protein ration. A problem may exist of preventing lysine destruction in the rumen so it can be absorbed in the intestines. Work has suggested that lysine is not deaminated by rumen microorganisms (61, 91); however, whether or not it retains its chemical identity in the rumen remains to be proven.

Corn gluten meal differs from soybean oil meal in nutrients other than soluble nitrogen and amino acids. Mineral matter, in particular, is lower in corn gluten meal. Although the influence of minerals in soybean oil meal cannot be ruled out entirely as being partially responsible for its superior value, this possibility seems rather slight. The in vitro cultural medium contained adequate amounts of all minerals known to influence rumen microbial activity (52, 58), and the semi-purified lamb rations contained all known required minerals in supposedly adequate amounts.

The interesting work of Matrone et al. (66) which clearly indicates a need for alkaline mineral compounds suggests that forms in which minerals are provided may be more critical than total amounts under certain conditions. The significance of these observations in relation to mineral matter in natural feeds requires further study. The excellent performance of the lambs fed soybean oil meal in all of the lamb feeding trials certainly is indicative of the adequacies of the semi-purified rations fed.

The results of early experiments in this study were interpreted to indicate that corn gluten meal did not contain protein quality factors present in soybean oil meal. Most of the lamb feeding trials in which the significance of the water soluble fraction of soybean oil meal was studied were conducted under this assumption, and corn gluten meal was used as the protein source in these rations. Although marked improvements in lamb performance resulted when this fraction of soybean oil meal was added, whether this was due to unidentified factors or simply available nitrogen was not answered. The in vitro rumen studies definitely indicated that unidentified factors were concentrated in this fraction, for these responses were produced in the presence of adequate urea nitrogen, and the stimulation from these factors diminished in the absence of adequate urea nitrogen.

The water soluble fraction of soybean oil meal definitely appeared to contain factors which have not been identified as specific requirements

for ruminant animals. One may surmise that these factors are requirements of the microbial population in the rumen. These factors have been primarily studied with in vitro microbial cultures; and as a result, their significance beyond the rumen is poorly understood. Whether improved lamb performance in these studies was the result of stimulated rumen function or stimulation beyond the rumen is not known. Bentley et al. (15) have postulated that such factors increase the rate of digestion but do not improve efficiency of digestion.

In light of the results of the final lamb trial, it appears that available nitrogen is more of a limiting factor in corn gluten meal than unidentified factors. Lack of response from the amino acids lysine and methionine and from soybean oil meal extract in the presence of urea may be interpreted in several ways. First, if amino acids were limiting in corn gluten meal, perhaps the microorganisms were able to synthesize adequate quantities of them from urea nitrogen. Second, and more probably, corn gluten meal may contain adequate unidentified factors. These may be available to the microorganisms only if sufficient available nitrogen is present to promote active microbial fermentation. The presence of urea supplied nitrogen for active fermentation, corn gluten meal was more extensively degraded, and the unidentified factors became available. The possibility also exists and is supported by the in vitro experiments that, for optimum response from unidentified factors, adequate available nitrogen is required.

It is, therefore, concluded from this work that protein quality is comprised of at least two separate identities, available nitrogen and factors unidentified.

SUMMARY

This work consisted of three distinct phases - determining the significance of soluble nitrogen in protein feeds for ruminants, measuring the merits of the water soluble fraction of soybean oil meal, and investigating the importance of readily available nitrogen and amino acid balance in a low quality protein feed.

The utilization of several protein feeds by rumen microorganisms was observed to be related in a general way to nitrogen soluble in rumen fluid. Corn protein and heat treated soybean oil meal were particularly low in soluble nitrogen, only slowly converted to ammonia by rumen microbial degradation, and ineffective as nitrogen sources for in vitro cellulose digestion by rumen microorganisms. Regular soybean oil meal, linseed meal, casein and purified soy protein were rapidly converted to ammonia and were suitable sources of nitrogen for in vitro rumen cellulose digestion.

Reduction of soluble nitrogen by heat treating ration ingredients did not affect lamb performance. Soybean oil meal, regular and heat treated, and linseed oil meal were of equal value in promoting growth of lambs fed semi-purified rations. Corn gluten meal was inferior to the above protein feeds.

The water soluble fraction of soybean oil meal consistently improved cellulose digestion in vitro by rumen microorganisms. Attempts

at characterizing the active factors in this fraction were less consistent and no definite conclusions can be drawn.

Corn gluten meal and soybean oil meal as protein sources in a semi-purified ration were compared in a digestibility and nitrogen balance trial, and cellulose digested by lambs fed corn gluten meal was significantly lower than cellulose digested by lambs fed soybean oil meal. No apparent differences in protein digestibility and nitrogen balance were detected. Lamb growth was favorably stimulated in three of four trials in which the water soluble fraction of soybean oil meal was incorporated in a semi-purified ration containing corn gluten meal as the major protein source.

Additions of lysine and methionine to corn gluten meal appeared to be of little value; however, the addition of urea to corn gluten meal greatly improved its value both in stimulating in vitro rumen cellulose digestion and in increasing lamb gains. The water soluble fraction of soybean oil meal was without effect on lamb performance when corn gluten meal and urea were combined as the nitrogen sources in the semi-purified ration. This was interpreted to indicate that readily available nitrogen is the first limiting property of corn gluten meal.

It was concluded that protein quality is comprised of at least two separate identities, available nitrogen and factors unidentified. The quality factors are concentrated in the water soluble fraction, and readily available nitrogen is necessary for the utilization of the unidentified factors.

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APPENDIX

Table 22. Composition of mineral mixture included in semi-purified ration

Constituent	Amount[a]
NaCl	8.70 lbs.
K_2HPO_4	11.20 lbs.
KCl	7.30 lbs.
$CaHPO_4 \cdot 2H_2O$	13.00 lbs.
$MgSO_4 \cdot 7H_2O$	7.16 lbs.
$CaSO_4 \cdot 2H_2O$	14.78 lbs.
$CaCO_3$	1.07 lbs.
$FeSO_4 \cdot 7H_2O$	0.50 lbs.
KI	12.712 gm.
$ZnSO_4 \cdot H_2O$	5.039 gm.
$CuSO_4 \cdot 5H_2O$	4.994 gm.
$CoSO_4 \cdot 7H_2O$	4.426 gm.
CaF_2	3.632 gm.
$MnSO_4 \cdot H_2O$	22.700 gm.

[a]Added to ration at the rate of 6.3 pounds per 100 pounds total ration.

Table 23. Composition of vitamin mixture included in semi-purified ration

Constituent	Amount[a] (grams)
Thiamine HCl	2.815
Riboflavin	4.495
Niacin	22.609
Pyridoxine HCl	2.815
Calcium pantothenate	22.609
Biotin	0.282
Folic acid	1.135
Menadione	2.815
Vitamin B_{12}	0.005
Inositol	45.400
Para-aminobenzoic acid	45.400
Quadrex "10"[b]	100.000
Alpha-tocopheryl acetate	10.000
Choline chloride	454.000
Stilbestrol	0.600
Corn starch	3825.020

[a]Added to ration at the rate of 454 grams per 100 pounds total ration.

[b]Contains 10,000 units vitamin A and 1,250 units vitamin D per gram.

Table 24. Analyses of variance of effects on lamb performance of heating protein ingredients to reduce nitrogen solubility

Source of variation	Df.	M. sq.	
		Weight gain	Feed consumption
Total	35	0.0043	0.1067
Treatment	5	0.0052	0.1475
Remainder	30	0.0042	0.1000

Table 25. Statistical analyses of effects on lamb performance of different protein feeds as nitrogen sources in semi-purified rations

Source of variation	Df.	M. sq.	
		Weight gain	Feed consumption
Total	23	0.0457	0.392
Treatment	3	0.1500	1.193
Remainder	20	0.0301	0.272

Shortest significant ranges

Average daily gain:

p:	(2)	(3)	(4)
R_p:	.21	.22	.23

Treatments:	4	1	3	2
Means:[a]	.28	.55	.60	.61

Average daily feed:

p:	(2)	(3)	(4)
R_p:	.63	.66	.68

Treatments:	4	1	2	3
Means:[a]	2.75	3.52	3.67	3.69

[a]Any two means not underscored by the same line are significantly different at the 0.05 level of probability.

Table 26. Analyses of variance of digestibility and nitrogen balance data obtained with lambs fed corn gluten meal or soybean oil meal as protein sources in semi-purified rations

Source of variation	Df.	M. sq.			
		Organic matter	Protein	Cellulose	% Intake N retained
Total	15	11.85	10.19	77.36	16.84
Treatment	1	62.88	13.95	481.80	5.67
Remainder	14	8.20	9.93	48.48	17.64

Table 27. Statistical analyses of performance data of lambs fed soybean oil meal or corn gluten meal supplemented with water soluble fraction of soybean oil meal

Source of variation	Df.	M. sq.	
		Weight gain	Feed consumption
Total	23	0.0191	0.234
Treatment	3	0.0259	0.543
Remainder	20	0.0180	0.188

Shortest significant ranges

Average daily gain:

p:	(2)	(3)	(4)
R_p:	.16	.17	.17

Treatments:	3	1	4	2
Means:[a]	.54	.62	.68	.68

Average daily feed:

p:	(2)	(3)	(4)
R_p:	.52	.55	.56

Treatments:	3	4	2	1
Means:[a]	3.75	3.83	4.19	4.39

[a]Any two means not underscored by the same line are significantly different at the 0.05 level of probability.

Table 28. Statistical analyses of effects on lamb performance of feeding water soluble fraction of soybean oil meal

Source of variation	Df.	M. sq. Weight gain	M. sq. Feed consumption
Total	23	0.0558	0.530
Treatment	3	0.2284	2.153
Remainder	20	0.0299	0.286

Shortest significant ranges

Average daily gains:

p:	(2)	(3)	(4)
R_p:	.21	.22	.22

Treatments:	1	2	3	4
Means:[a]	-.02	.11	.20	.45

Average daily feed:

p:	(2)	(3)	(4)
R_p:	.64	.68	.69

Treatments:	1	2	3	4
Means:[a]	1.71	1.90	2.20	3.06

[a]Any two means no underscored by the same line are significantly different at the 0.05 level of probability

Table 29. Statistical analyses of effects on lamb performance of ration additions of soybean oil meal water extract in dried and liquid forms

Source of variation	Df.	M. sq.	
		Weight gain	Feed consumption
Total	29	0.0315	0.1285
Treatment	3	0.1002	0.1439
Remainder	26	0.0236	0.1267

Shortest significant ranges

Average daily gain:

p:	(2)	(3)	(4)
R_p:	.16	.17	.18

Treatments:	1	2	3	4
Means:[a]	.20	.38	.42	.47

Average daily feed:

p:	(2)	(3)	(4)
R_p:	.38	.40	.41

Treatments:	1	4	3	2
Means:[a]	1.73	1.94	2.02	2.04

[a]Any two means not underscored by the same line are significantly different at the 0.05 level of probability.

Table 30. Statistical analyses of effects on lamb performance of ration supplementation with several nitrogenous substances

Source of variation	Df.	M. sq. Weight gain	M. sq. Feed consumption
Total	55	0.0343	0.211
Treatment	6	0.1233	0.293
Remainder	49	0.0234	0.201

Shortest significant ranges

Average daily gain:

p:	(2)	(3)	(4)	(5)	(6)	(7)
R_p:	.15	.16	.17	.17	.17	.18
Treatments:	5	6	4	3	1	2	7
Means:[a]	-.02	-.01	.05	.06	.14	.16	.35

Average daily feed:

p:	(2)	(3)	(4)	(5)	(6)	(7)
R_p:	.45	.47	.49	.50	.51	.52
Treatments:	6	5	4	3	1	2	7
Means:[a]	1.32	1.43	1.49	1.55	1.69	1.70	1.88

[a]Any two means not underscored by the same line are significantly different at the 0.05 level of probability.

Table 31. Statistical analyses of effects on lamb performance of ration additions of readily available nitrogen and amino acids

Source of variation	Df.	M. sq.	
		Weight gain	Feed consumption
Total	29	0.0256	0.162
Treatment	4	0.0616	0.345
Remainder	25	0.0199	0.133

Shortest significant ranges

Average daily gain:

p:	(2)	(3)	(4)	(5)
R_p:	.17	.18	.18	.19

Treatments:	1	5	4	3	2
Means:[a]	.13	.27	.35	.36	.38

Average daily feed:

p:	(2)	(3)	(4)	(5)
R_p:	.43	.45	.47	.48

Treatments:	1	5	4	3	2
Means:[a]	2.15	2.48	2.49	2.72	2.75

[a]Any two means not underscored by the same line are significantly different at the 0.05 level of probability.